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Genetic Overlap between Type 2 Diabetes and Depression

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Abstract

Background

An association between type 2 diabetes (T2DM) and depression has been reported in epidemiological studies. The mechanisms underlying the T2DM-depression link remain unclear. One possible question is whether the co-occurrence of T2DM and depression is due to common genetic and/or common environmental vulnerabilities. A genetic overlap between T2DM and depression will provide evidence supporting a common biological pathway to both disorders.

Method

This thesis applied three methodological approaches: i) structural equation modelling of twin data, ii) polygenic score analysis and iii) linkage disequilibrium score regression using genome-wide association studies (GWAS) data. For the first approach, the primary dataset was the Swedish Twin Registry with replication in the Danish Twin Registry and Colombo Twin and Singleton Study (COTASS-2). For the second and third approach, the population cohorts deCODE and UK Biobank, and the Psychiatric Genomics Consortium Major Depressive Disorder (PGC-MDD-29) dataset were used.

Result

In the twin studies, a genetic overlap between T2DM and depression was observed in the Swedish Twin Registry and the finding was replicated in the Danish Twin Registry and COTASS-2. In the GWAS datasets, T2DM-polygenic scores were not a major contribution to depression nor depression-polygenic scores for T2DM in both deCODE and UK Biobank. A small, negative but statistically significant association was observed

between feelings of guilt/worthlessness and T2DM-polygenic scores in the PGC-MDD-29 dataset.

Conclusion

Twin studies have suggested a genetic overlap between T2DM and depression. There are many reasons to explain the discrepancies in findings between twin studies and GWAS. Clarifying the shared heritability between these two complex traits is an important next step while gene-environment interaction is an area that needs to be explored, given genotypes can affect an individual's responses to the environment and environment can differentially affect genotypes expression.

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While I was a psychiatry trainee, I was fortunate to meet Professor Janet Treasure. She has been instrumental in encouraging me to partake on this academic journey and have been an inspiration ever since. She is simply the best.

This thesis is based on phenotypic data from the Swedish Twin Registry, Danish Twin Registry and Colombo Twin and Singleton Study Phase 2, and the genomic data from the Psychiatric Genomics Consortium Major Depressive Disorder study, deCODE and UK Biobank cohorts. I would like to thank all participants in contributing their time. I am also extremely grateful for the inputs and supports I have received from our collaborators and members of their teams.

I would also like to thank my family and friends for being on this thrilling academic journey with me. No words can ever truly express my appreciation of their love and support, from R codes troubleshooting to home cooked meals and late night cocktails. What would I do without them all?

“I can no other answer make, but, thanks, and thanks, and ever thanks.” ~ William Shakespeare, Twelfth Night

Author Declaration

The work presented in this thesis was undertaken as part of my clinical research fellowship funded by the Novo Nordisk UK Research Foundation.

Prior to commencing my PhD, the phenotypic data for the Swedish Twin Registry, Danish Twin Registry, Colombo Twin and Singleton Study Phase 2, and the genomic data for the Psychiatric Genomics Consortium Major Depressive Disorder study, deCODE and UK Biobank cohorts were already collected. I was responsible for preparing the phenotypic and genomic data for all statistical analyses in this thesis, with support from Dr Frühling Rijsdijk, Professor Cathryn Lewis and Professor Khalida Ismail.

To the best of my knowledge, the work presented in this thesis is original and my own work, except where acknowledged in the text.

Presentations and publications arising from this thesis as of 31st July 201

Oral Presentations

- 2016 Genetic overlap of diabetes and depression: an overview, Royal College of Psychiatrists International Congress, UK
- 2016 Genetic overlap of diabetes and depression, North Europe Young Diabetologist Annual Meeting, Denmark
- 2016 Genetic overlap of depression and physical health disorders, American Depression and Anxiety Conference, USA

Poster Presentations

- 2017 Effect of waist-to-hip ratio on the association between type 2 diabetes and depression, Academy of Medical Science Spring Meeting, UK
- 2016 Examining the type 2 diabetes and depression link using polygenic risk scores, European Human Genetics Conference, Spain
- 2015 Polygenic risk scores in type 2 diabetes and depression, American Diabetes Association Scientific Session, USA
- 2015 The genetic overlap of type 2 diabetes and depression, European Congress of Psychiatry, Austria [Best Poster Award]
- 2015 Gender differences in the genetic overlap between type 2 diabetes and depression, Diabetes UK Professional Conference, UK

Publication

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List of Abbreviations

COTASS-2	Colombo Twin and Singleton Study Phase 2
DIAGRAM	Diabetes Genetic Replication And Meta-analysis Consortium
DZ	Dizygotic
GCTA	Genome-wide Complex Trait Analysis
GWAS	Genome-wide association studies
LD	Linkage disequilibrium
MZ	Monozygotic
PC	Principal component
PCA	Principal component analysis
PGC-MDD-1	Psychiatric Genomics Consortium-Major Depressive Disorder Stage 1
PGC-MDD-29	Psychiatric Genomics Consortium Major Depressive Disorder Phase 2
P_T	p -value threshold
SNP	Single nucleotide polymorphisms
T2DM	Type 2 diabetes

Chapter 1

Introduction

“Heaven knows we need never be ashamed of our tears, for they are rain upon the blinding dust of earth, overlying our hard hearts. I was better after I had cried, than before - more sorry, more aware of my own ingratitude, more gentle.”

~ Charles Dickens, Great Expectations

1.1 Introduction

Type 2 diabetes (T2DM) and depression are both common disorders with a lifetime prevalence of 8.3% and 16% respectively (International Diabetes Federation, 2012; Kessler et al., 2003). In the UK, T2DM affects 7% of the population while depression affects 20% (Holman, Forouhi, Goyder, & Wild, 2011; Office for National Statistics, 2016). They were respectively ranked as the seventh and second leading causes of disability adjusted life years in a global burden of disease study conducted in 2013 (Global Burden of Disease Study Collaborators, 2015). The associated costs to the National Health Service is staggering, with one study estimating the direct cost to be £8.8 billion for T2DM in 2010, and £1.7 billion for depression in England in 2007 (Hex, Bartlett, Wright, Taylor, & Varley, 2012; McCrone, 2008). The respective figures are projected to be £15.1 billion by 2035 and £3 billion by 2026 (Hex et al., 2012; McCrone, 2008).

1.2 What is T2DM?

T2DM is a chronic medical disorder occurring when there is an ineffective use of insulin produced by the body, and/or a progressive reduction in insulin production. The number of people with diabetes worldwide has increased from 108 million in 1980 to over 400 million in 2014, with a global prevalence (age-standardized) rising from 4.7% in 1980 to 8.5% in 2014 among adults (NCD Risk Factor Collaboration, 2016). Prevalence rates during this period has either increased or at best remained unchanged in every country, with the rise being most noticeable in middle- and low-income countries (Mathers & Loncar, 2006). The largest numbers of people with diabetes were estimated for the South-East Asia and Western Pacific Regions, and together they account for approximately half of the diabetes cases in the world.

Risk factors for T2DM include obesity, hypertension, hyperlipidaemia, poor dietary habits and physical inactivity. The prevalence of T2DM has increased secondary to the obesity epidemic (Holman et al., 2011). One of the consequences of poorly uncontrolled diabetes is raised blood glucose level (hyperglycaemia). This can lead to long-term macrovascular and microvascular complications such as an increased risk of myocardial infarction, ischaemic stroke, retinopathy and neuropathy.

Achieving and maintaining optimal glycaemic control is therefore essential for individuals with T2DM. A review of 26 general practices in South London reported the median proportion of diabetic patients with a HbA_{1c} (a measure of glycated haemoglobin which is indicative of average blood glucose levels over 3 previous months) of ≤ 57.4 mmol/mol was 59.0% (Gulliford, Ashworth, Robotham, & Mohiddin, 2007; Mattila & de Boer, 2010). Diabetes management is, however, complex as optimising glycaemic control is only one aspect of care. Timely prevention, detection and management of complications are equally important in improving the quality of life for an individual. The latest recommendations from the National Institute for Health and Care Excellence (NICE) include patient education, dietary advice, blood pressure management and drug treatment (Gatineau et al., 2014; McGuire, Longson, Adler, Farmer, & Lewin, 2016). They also emphasise the importance of involving and supporting patients in decisions about their individual blood glucose target. NICE recommends individuals with T2DM should receive annual health checks to monitor and manage their condition, in order to reduce risks of diabetes-related complications.

1.3 What is depression?

According to the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM), the core symptoms of depression include low mood and anhedonia (loss of interest in activities once enjoyed)

(American Psychiatric Association, 2013). It can lead to various psychological and physical difficulties, such as loss of appetite, insomnia, fatigue, poor concentration, feelings of guilt/worthless and suicidality. Depression can also have significant impacts on an individual's ability to function at work and at home. The severity of depression is determined by the number, nature and severity of symptoms, as well as their effects on functional impairment. Symptoms must be present for at least two weeks for a diagnosis of depression.

There are wide variabilities in prevalence estimates for depression across the world. The first cross-national study was conducted in the early 1990s. It reported lifetime prevalence estimates of depression ranging from 1.5% (Taiwan) to 19.0% (Beirut), and twelve-month prevalence estimates ranging from 0.8% (Taiwan) to 5.8% (Christchurch, New Zealand) (Weissman et al., 1996). A subsequent comparison in the early 2000s reported lifetime prevalence estimates of depression from 1.0% (Czech Republic) to 16.9% (USA), and twelve-month prevalence estimates ranging from 0.3% (Czech Republic) to 10% (USA) (Andrade et al., 2003). The cross-national differences observed has been attributed to be due to a combination of definition of depression, instruments used and study design factors (Kessler & Bromet, 2013).

Depression remains a common psychiatric disorder worldwide, with the World Health Organisation currently ranking it as the fourth leading cause of disability worldwide (Murray & Lopez, 1996a) and projecting it as the second leading cause by 2020, it will be the second leading cause (Murray & Lopez, 1996b). It is usually first presents in primary care settings (Goldberg & Huxley, 1980). Depression is, however, often undetected and untreated, resulting in considerable service and societal costs. The first onset of depression is during the mid-20s, with a female predominance (Seedat et al., 2009).

Risk factors for depression include genetic and environmental factors, such as socioeconomic status, childhood adversities and experience

of recent negative life events (Otte et al., 2016). The clinical course of depression is often chronic, or remitting and relapsing, with residual symptoms and functional impairment being present between episodes. The key goal of treatment is complete remission (the absence of disease), as this is associated with a reduced likelihood of relapse. The main modality of treatment for depression is psychopharmacology and psychotherapy, with most guidelines recommending a combination of medication and psychotherapy for individuals with severe depression (National Institute for Health and Care Excellence (2016). A substantial number of patients may still remain unwell after several treatment attempts (Rush et al., 2006).

The lack of clinical effectiveness observed with treatment for depression, in addition to having no established biomarker in the daily clinical setting, could reflect in part the heterogeneous nature of depression (Kunugi, Hori, & Ogawa, 2015). This is further complicated by the number of depressive symptoms being included in the DSM. For example, 227 or more different symptom combinations are possible for an individual to meet DSM-IV or DSM-5 diagnostic criteria for depression (Galatzer-Levy & Bryant, 2013). Substantial heterogeneity is therefore observed in depression from symptom clusters, clinical course to response to pharmacological treatment (Ghaemi & Vohringer, 2011). It has been suggested specific depression subtypes are associated with different biological correlates (Lamers et al., 2013). Adopting a categorical classification based solely on phenomenology might hinder a more comprehensive understanding of its complex pathophysiology.

1.4 What is the T2DM and depression association?

Both cross-sectional and longitudinal studies have reported bidirectional association between T2DM and depression (Chen, Chan, Chen, Ko, & Li, 2013; Golden et al., 2008), with up to a 60% increased risk

for developing T2DM in individuals with depression; and in the reverse direction, 15% for incidental depression in those with T2DM (Mezuk, Eaton, Albrecht, & Golden, 2008; Rotella & Mannucci, 2013). The T2DM-depression link has been associated with adverse effects on diabetes outcomes including suboptimal glycaemic control, greater complications and higher rates of mortality. In addition, an association has been observed between depression and all stages of the clinical course of T2DM from insulin resistance to mortality (De Groot, Anderson, Freedland, Clouse, & Lustman, 2001; Kan et al., 2013; Katon et al., 2005; Lustman et al., 2000).

The underlying pathogenesis of the T2DM and depression association is complex and likely to arise from interplay of both genetic and environmental factors. Female sex, low socioeconomic status, social isolation, smoking, the presence of diabetes complications, duration of diabetes and persistence of poor glycaemic control have all been attributed as risk factors for depression specific to diabetes (E., Hermanns, & Nouwen, 2010; Leone, Coast, Narayanan, & de Graft Aikins, 2012; Roy, Lloyd, Parvin, Mohiuddin, & Rahman, 2012; Shehatah, Rabie, & Al-Shahry, 2010). Behavioural and lifestyle factors such as physical inactivity and poor self-care have, on the other hand, been implicated as mediating factors in elevating the risk of diabetes in individuals with depression (Golden et al., 2008). In addition, obesity, stressful life events, perceived burden of chronic illnesses, hypercortisolism and disturbed immune functions have been attributed to contribute to the T2DM-depression association (Kan et al., 2013; Renn, Feliciano, & Segal, 2011).

A question of interest is whether the association between T2DM and depression is predominantly due to shared genetic and/or environmental vulnerability. The evidence for a genetic component to T2DM is well-established, including observations from twin studies, linkage studies and genome-wide association studies (GWAS) (Barroso, 2005; Fuchsberger et

al., 2016). A recent meta-analysis estimates heritability for T2DM to be 72% (95% confidence interval (95% CI): 61-78% (Willemsen et al., 2015)).

The genetic basis of depression is more difficult to determine, with twin studies estimating the heritability at about 37% (95% CI: 31%-42% (Sullivan, Neale, & Kendler, 2000)). Two studies have, however, reported higher heritability in females than in males (40% versus 30%; 42% versus 29% respectively) and sex-specific genetic effects (Kendler, Gardner, Neale, & Prescott, 2001; Kendler, Gatz, Gardner, & Pedersen, 2006). Depression is further complicated by its high clinical heterogeneity. There is suggestion that gene-environment interaction may influence the risk for depression, although this effect was not replicated in a meta-analysis (Risch et al., 2009).

T2DM and depression share common risk factors. This observation could be purely the result of coincidence, or because the two disorders share a common biological pathway. For example, people with diagnostic depression have increased levels of inflammation, hyperactivity in the hypothalamic-pituitary adrenal (HPA) axis and sympathetic nervous system. This is also observed in people with T2DM, suggesting a common biological pathway between T2DM and depression. Other findings include a tryptophan hydroxylase polymorphism being associated with both metabolic disorders, and insulin resistance in individuals with depression (Chiba et al., 2000; Kloiber et al., 2010). In addition, inflammatory markers, such as C-Reactive Protein, have been associated with depressive symptoms in individuals with newly diagnosed T2DM (Laake et al., 2014). It is therefore possible the T2DM-depression association can in part be due to shared biological pathways.

T2DM and depression are global public health concerns. Finding a genetic overlap between T2DM and depression would provide more direct evidence that the epidemiological association observed was due to biological factors. It would also advance our knowledge of their pathogenesis, contribute to the search for subtypes of depression, and

adapt epidemiological methods for multi-morbid conditions. Although predicting genetic liability for complex traits is currently of limited clinical value, genetic profiling may one day allow us to stratify patients based on outcomes. For example, identifying individuals with T2DM who are at high risk for developing depression would allow appropriate interventions to be recommended. In the long term, a greater knowledge base about T2DM and depression will enable us to develop more effective treatments for patients with both conditions and to study why depression is common in other chronic conditions such as obesity. It is therefore important to review the current literature on the genetic basis of the T2DM-depression association.

1.5 Evidence from family studies

Family history is a useful first approach in capturing the joint contribution of genetic and environmental factors to a condition, or the comorbidity between conditions (familial effects). To date, there has been one family study of T2DM and depression published (Pravin, Malhotra, Chakrabarti, & Dash, 2006). It is a cohort study based in India, with a sample of 50 patients of T2DM and 481 of their first-degree relatives. The morbid risks for depression in first-degree relatives of probands of T2DM were found to be similar to the general population in other studies. There was, however, no comparison group, and individuals with any lifetime history of psychiatric disorders were excluded, leading to a low prevalence of depression (1.0%). Another similar study reported that the number of generations of diabetes in the family as a good predictor of child depressive symptoms (Irving et al., 2008). Family studies do not, however, differentiate between which factors contribute to first-degree family members being similar, since relatives share both genes and environment. Any familial effect observed could therefore be due to both genetic and/or environmental influences.

1.6 Evidence from twin studies

Twin studies provide a unique approach in investigating genetic influences on complex traits or disorders; as twins are matched for age, genetic factors and a range of covariates in their shared environment, such as parenting style and education. Twin study uses the ratio of disease concordance between monozygotic (MZ) and dizygotic (DZ) twins to estimate the most likely effects of genetic (heritability), common and individual-specific environmental factors. In simple terms, the method uses the ratio of cross-disorder concordance between MZ and DZ twins to approximate the genetic and environmental correlations between disorders. Genetic correlation estimates the degree to which genetic factors affect both disorders, and is independent of their individual heritability estimates. A high genetic correlation does not therefore imply a high impact of genes on the observed correlation between the conditions, and the actual heritability estimates for each disorder needs to be taken into account.

There have been two twin studies investigating common genetic and environmental vulnerability to diabetes and depression. The Vietnam Era Twin Study of Aging consists of MZ and DZ twins who served in the United States military during the Vietnam era (1965-1975; $n=1237$) (Scherrer et al., 2011). It reported a moderate genetic correlation between T2DM and depression, with a broad confidence interval (0.19 (95% CI: 0-0.46)). The finding is difficult to interpret, given the sample was restricted to males aged 50-59 who were involved in military service. The Screening Across the Lifespan Twin Study consists of MZ and DZ twins aged 40 years or older recruited from the Swedish Twin Registry ($n=37043$) and concluded there was significant environmental correlation (0.54 (95% CI: 0.02-0.88)) between T2DM and depression (Mezuk, Heh, Prom-Wormley, Kendler, & Pedersen, 2015). Both studies do not support a common genetic pathway hypothesis. Higher rates of co-morbid T2DM and depression have been observed in females compared to males (Ali, Stone, Peters, Davies, &

Khunti, 2006; Alonso-Moran, Satylganova, Orueta, & Nuno-Solinis, 2014; R. J. Anderson, Freedland, Clouse, & Lustman, 2001), with a meta-analysis reporting a higher prevalence of depression in females with T2DM (23.8%) than males (12.8%; (Ali et al., 2006). Clearly, twin modelling incorporating sex differences is needed.

1.7 Evidence from molecular genetic studies

The revolution in genomics – the ability to rapidly sequence DNA and conduct GWAS - has uncovered many genetic contributions to the development and progression of complex traits. To date, over 120 single nucleotide polymorphisms (SNPs) predisposing to T2DM and diabetes-related traits have been discovered (Prasad & Groop, 2015). These variants are associated with a small risk of developing T2DM (odd ratios (OR): 1.0 - 1.2), and in sum capture a small proportion of the genetic component of the disease. The strongest association is observed in the wnt signalling pathway member, transcription factor-7-like 2 on chromosome 10 (Grant et al., 2006; Kildemoes, Sorensen, & Hallas, 2011; S. Purcell, 2002). It is 1.5 times more common in patients than in controls, conferring an approximately 40% increased risk for T2DM. Rare variants have been associated with T2DM, but a recent analysis using empirical and stimulated data suggests rare variants are unlikely to be an important source of contribution to the heritability of T2DM, in comparison to common variants (Fuchsberger et al., 2016).

For depression, GWAS have been unable to identify any reproducible associations signal until recently. A meta-analysis did not identify any genome-wide significant finding (Ripke et al., 2013), with the authors attributing the negative finding to the inherent heterogeneity of the phenotypes, and insufficient power due to the high prevalence rate of depression in the general population. Fifteen novel SNPs for depression have since been identified in individuals of European descent using self-report data (Hyde et al., 2016), whereas a study in Chinese females with

severe depression has identified two loci that are rare in Europeans (CONVERGE, 2015). In addition, a rare missense Asn396Ser mutation in the endothelial lipase gene has recently been implicated in the pathogenesis of depressive symptoms in a large population-based cohort (Amin et al., 2016).

A range of T2DM-related SNPs have been suggested to be associated with depression. For example, the Pro12Ala variant of the peroxisome proliferative-activated receptor γ 2 gene has been implicated in inflammation, depression, type 1 and 2 diabetes (Eftychi et al., 2004; Ji-Rong et al., 2009). A locus involved in the circadian gene CRY2 has been associated with both T2DM (Dupuis et al., 2010) and major depressive disorder with seasonal pattern (Lavebratt et al., 2010). A small case control study has found individuals with depression have reduced expression of FADS1, a gene involved in long-chain polyunsaturated fatty acids biosynthesis and T2DM, compared to controls (McNamara & Liu, 2011). Depressive behaviours have also been observed in adult mice with Insulin-like growth factor-1 knock-out, a gene also implicated in T2DM (Mitschelen et al., 2011). In addition, SNPs associated with leptin, a peptide hormone from adipose tissue, has been linked with response to antidepressant treatment (Kloiber et al., 2013) and T2DM (Hara et al., 2014).

Genotypes for α -2A adrenoceptor (ADRA2A) and melatonin receptor 1B (MTNR1B) have been associated with raised plasma glucose, a T2DM-related trait (Dupuis et al., 2010) (Manning et al., 2012). The ADRA2A genotype has been suggested as a predictor of treatment outcome in people with depression (Kato et al., 2015), and sex-difference has been reported between the activity of the HPA-axis and the ADRA2A genotype (Haefner et al., 2008). Genetic polymorphisms and mRNA expression of the MTNR1B, on the other hand, has been demonstrated to play a significant role in patients with recurrent depression (Galecka et al., 2011). Studies have also indicated a role of the FTO genes in T2DM and body mass index

(BMI) (Frayling et al., 2007; Mahajan et al., 2014) and a meta-analysis showed a significant inverse association between the FTO rs9939609 A variant and depression (Samaan et al., 2013). Findings from molecular genetic studies provide some tentative evidences of the potential of T2DM-predisposing genes on depression.

1.8 Evidence from statistical genetics using GWAS

Both T2DM and depression are likely to be polygenetic in nature, with hundreds of susceptible alleles of small effects. T2DM risk alleles may contribute small increments in depression risk, and vice versa. A polygenic score approach allows the cumulative effect of multiple T2DM genetic risk variants on depression status to be analysed, clarifying whether a genetic predisposition to T2DM might be related to depression and vice versa. In parallel to this thesis, the T2DM-depression association was examined in two GWAS datasets (Clarke et al., 2016; Samaan et al., 2015). Both concluded that there is little evidence of a genetic overlap between T2DM and depression. The EpiDREAM study examined the unidirectional association between 20 T2DM SNPs and depression case-status in 17404 individuals who were at risk for developing T2DM (Samaan et al., 2015). The Generation Scotland study examined the shared aetiology between T2DM and depression using polygenic scores and Mendelian Randomization in a population cohort of 21516 individuals (Clarke et al., 2016).

There are, however, caveats with both studies, making their findings difficult to generalise. The EpiDREAM study only examined 20 SNPs associated with T2DM, and over 120 SNPs have been identified with T2DM and related traits at GWAS significant level (Prasad & Groop, 2015). Thus, 20 SNPs are likely to capture only a very small proportion of the genetic component of T2DM. The Generation Scotland study, on the other hand, only has 915 individuals with T2DM. Further research needs

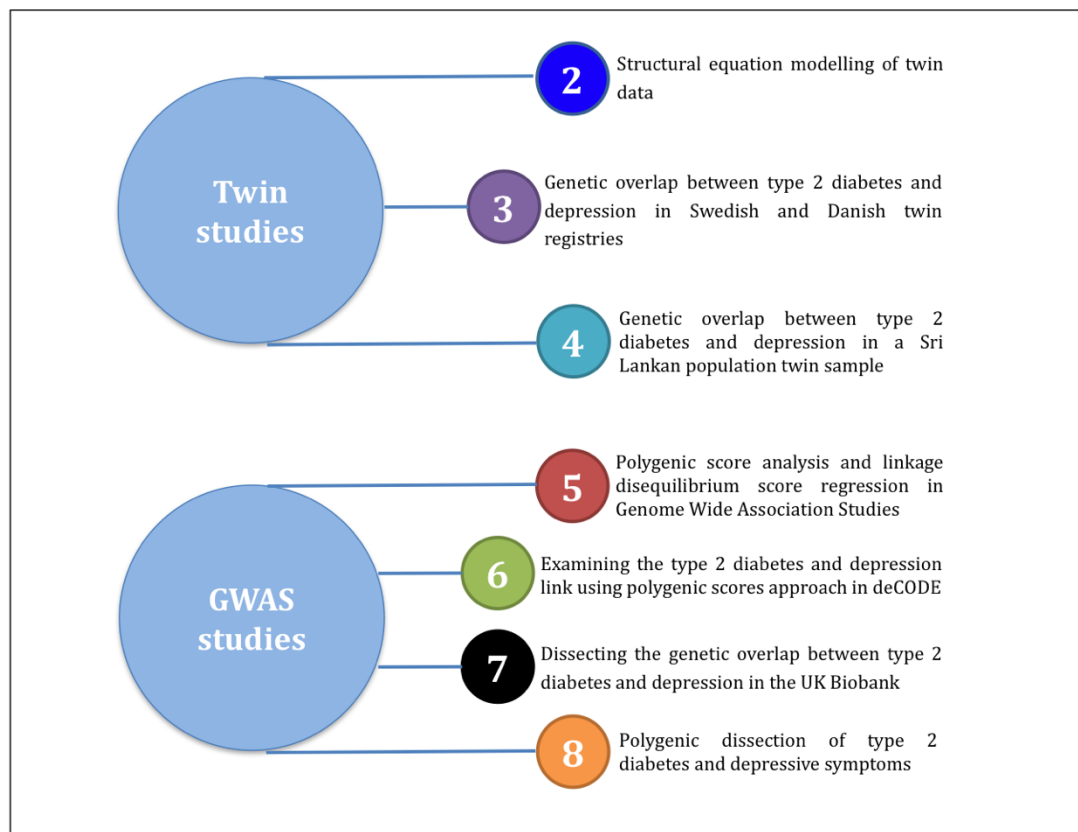
to be conducted in cohorts with larger sample size and non-Western populations. In addition, a recent study has demonstrated a genetic overlap between BMI-polygenic scores and atypical depression, defined by increased appetite or weight, but not typical depression . Given the heterogeneity of depression, the degree of variance in depression subtypes which can be explained by the additive effects of common SNPs associated with T2DM needs to be explored.

Linkage disequilibrium (LD) score regression has been applied in one study to estimate genetic correlations among 24 complex traits, including T2DM and depression. It uses the summary statistics from Diabetes Genetic Replication And Meta-analysis Consortium (DIAGRAM) and Psychiatric Genomics Consortium-Major Depressive Disorder Stage 1 (PGC-MDD-1) and estimated a non-significant, positive genetic correlation between T2DM and depression (r_g (standard error): 0.051 (0.117), $p=0.666$). If there is genetic heterogeneity between cohorts, estimates from LD score regression analysis can be biased downwards. Given both DIAGRAM and PGC-MDD-1 consist of multiple cohorts, it will be useful to apply the LD score regression in one large population cohort to estimate the genetic correlation between T2DM and depression.

1.9 Overview of thesis

The objective of this thesis was to identify whether there is a genetic overlap between T2DM and depression using three complementary approaches: i) structural equation modelling in twin data, ii) polygenic score analysis on case-control data and iii) LD score regression on summary-level GWAS data (Figure 1-1). The statistical methods involved are discussed in chapters 2 and 5 respectively.

Figure 1-1 PhD thesis chapter summary



For the first approach, I explored the T2DM-depression link using two large, population-based Scandinavian twin registries (Swedish and Danish twin registries; chapter 3). To examine whether our findings are generalizable to non-Western populations, I applied structural equation modelling to the Colombo Twin and Singleton Study Phase 2 (COTASS-2; chapter 4), given that heritability only refers to a particular population and environment.

For the second and third approach, we focused on GWAS datasets, as consistency of a finding across different sample populations and methodologies is essential in order to reduce sampling error and for a more robust conclusion to be inferred. The population-based deCODE cohort was used as an exploratory study to examine the genetic overlap between T2DM and depression using the polygenic score approach (chapter 6). I also performed secondary analysis, as it is unclear whether the genetic overlap between T2DM and depression differ with age, sex and BMI.

I then validated these findings in the UK Biobank cohort (chapter 7). I also estimated the genetic correlation between T2DM and depression using LD score regression in the UK Biobank cohort. In order to dissect the heterogeneous nature of depression, I then investigated the association between specific depressive symptoms and T2DM-polygenic scores in the Psychiatric Genomics Consortium Major Depressive Disorder Phase 2 (PGC-MDD-29) study (chapter 8).

Given the inherent heterogeneity of the studies included in this thesis, a comprehensive description of the various definitions used for i) T2DM and depression phenotype and ii) T2DM and depression polygenic scores is summarised in Table 1.1.

Table 1.1 Summary of measures used by thesis chapter.

	Type 2 diabetes definition	Depression definition	Type 2 diabetes genetic risk scores definition	Depression genetic risk scores definition
Chapter 3				
Swedish twin registry	Primary diagnosis of type 2 diabetes ((International Classification of Disease (ICD) 8 th , 9 th and 10 th edition); hospital registry data	Primary diagnosis of depression (ICD 8 th , 9 th and 10 th edition); hospital registry data	NA	NA
Danish twin registry	Primary diagnosis of type 2 diabetes (ICD 8 th and 10 th edition); hospital registry data	Primary diagnosis of depression (ICD 8 th and 10 th edition); hospital registry data	NA	NA
Chapter 4				
Colombo Twin and Singleton Study	Primary diagnosis of type 2 diabetes; self-report	Beck Depression Inventory; self-report	NA	NA
Chapter 6				
deCODE	Primary diagnosis of type 2 diabetes; clinical records	Primary diagnosis of lifetime depression (Diagnostic and Statistical Manual of Mental Disorder (DSM) 4 th edition and ICD 10 th edition); structured diagnostic interview or clinician administered DSM checklist	Diabetes Genetic Replication And Meta-analysis Consortium (DIAGRAM) Stage 1	Psychiatric Genomics Consortium Major Depressive Disorder (PGC-MDD) Stage 1 study

Chapter 7				
UK Biobank	A combination of i) medical diagnosis, ii) age at first diagnosis and iii) use of oral anti-diabetic medications; self-report to define type 2 diabetes and differentiate from type 1 diabetes.	A combination of i) primary diagnosis of depression; (ICD 10); inpatient hospital episode data or ii) previous visit to a General Practitioners/psychiatrist for stress, anxiety or depression, and at least one period of depression/anhedonia lasting at least two weeks; self-report	DIAGRAM Stage 1	PGC-MDD Phase 2 (PGC-MDD-29) study
Chapter 8				
PGC-MDD-29	NA	Primary diagnosis of lifetime depression (DSM-IV); structured diagnostic interview	DIAGRAM Stage 1	NA

1.10 Summary

T2DM and depression are among the leading causes of morbidity and mortality globally. The resultant societal costs of medical care and lost productivity have major public health impacts. A better understanding of the mechanisms underlying the association is therefore of high clinical and scientific relevance. There are real opportunities for developing novel therapeutic modalities, while facilitating the development of stratified medicine for people with T2DM and depression, by contributing to the research for targeted treatment of T2DM subtypes that have worse prognosis. Unravelling the genetic predisposition to T2DM and depression is a major challenge but presents an exciting opportunity to further our understanding of the genetic architecture and biological mechanisms underlying T2DM and depression across different populations.

Chapter 2

Structural equation modelling of twin data

“Melancholy is the happiness of being sad.”

~ Victor Hugo, The Toilers of the Sea

2.1 Overview

The epidemiological association of type 2 diabetes (T2DM) and depression can be attributed to genetic and environmental sources. Advanced statistical methods using genetically sensitive data allow us to estimate the extent to which the two disorders are associated due to overlapping genetic and/or environmental factors. The classical twin method is a valuable study design in behavioural genetics, as monozygotic (MZ) and dizygotic (DZ) twins provides a natural window into the aetiology of a trait and the overlap between traits. In this chapter, we will focus on the principles underlying structural equation modelling in twin data.

2.2 The classical twin design

The classical twin method is designed to estimate the relative genetic and environmental contributions to the variance of a trait/disorder (Martin, Boomsma, & Machin, 1997). It has three assumptions; i) MZ twins share 100% of their genes in common while DZ twins share on average 50% of their segregating genes (additive genetic effects), ii) MZ and DZ twins are correlated for shared environmental influences to the same extent (equal environment assumption) and iii) mating in the population occurs at random (non-assortative mating). Therefore, if a behaviour is genetically influenced, a greater similarity in that behaviour among MZ than DZ pairs would be observed.

In the most basic twin model, phenotypic variation (V_P) is assumed to arise from three sources: i) additive genetic factors (A), ii) common environmental factors (C), and iii) unique environmental factors (E). C serves to make twins (and all members of the same family) more similar to one another, while E tends to make the individuals in a family (or in twin pairs) less similar. Twin data allows us to estimate the heritability of complex traits.

2.3 What is heritability?

Heritability (h^2) is defined as the proportion of V_P due to variation in genetic factors (V_G ; $h^2 = \frac{V_G}{V_P}$), that is the proportion of individual differences for a trait in a certain population due to genetic differences between individuals. Genetic factors consist of several subcategories, including additive variance (V_A), dominance variance (V_D), and epistatic variance (V_I ; $V_G = V_A + V_D + V_I$). Together, they reflect heritability in the broadest sense (broad-sense heritability). The narrow-sense heritability includes only genetic variation due to additive genetic values (V_A ; $h^2 = \frac{V_A}{V_P}$). Similarly, the proportion of V_P due to variation in common environmental factors (V_C) and unique environmental factors (V_E) are summarised as $c^2 = \frac{V_C}{V_P}$ and $e^2 = \frac{V_E}{V_P}$ respectively.

2.4 Univariate Genetic Analysis

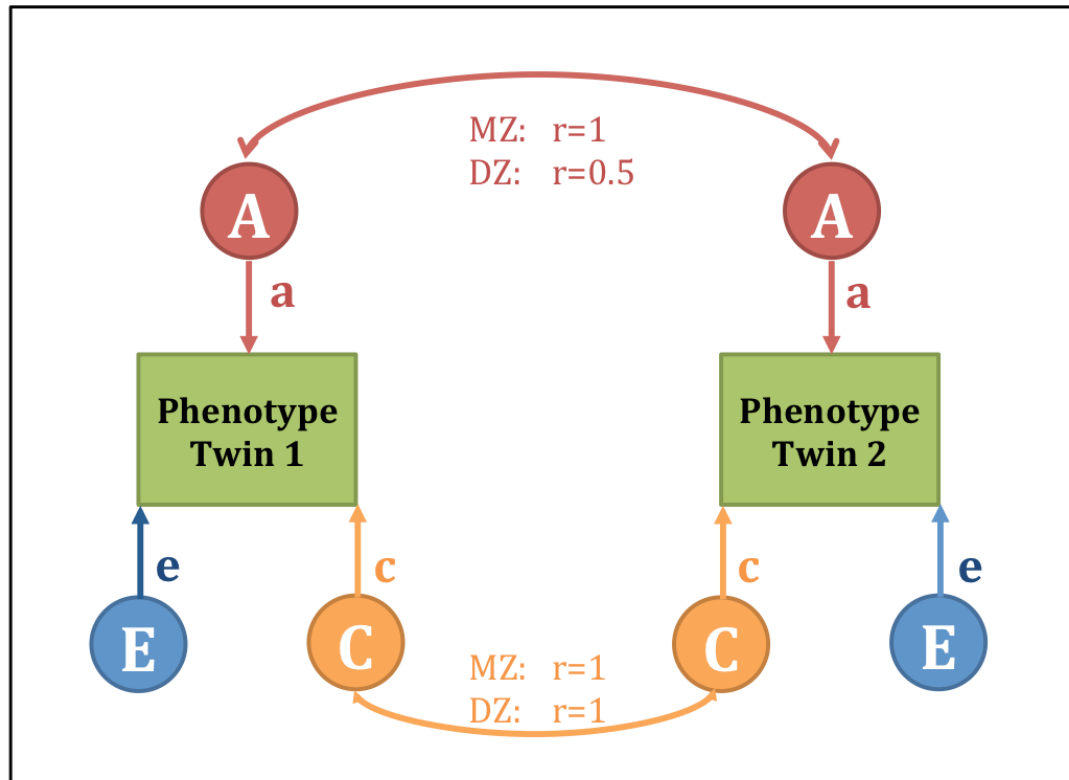
The equal environment assumption underlying the classic twin design implies that any excess of similarity between MZ and DZ twins is due to the greater proportion of genetic factors A being shared by MZ twins. Therefore, if the MZ correlation is twice as large as the DZ correlation ($r_{MZ} > 2r_{DZ}$), there is evidence of additive genetic effects. If the MZ correlation is less than twice as large as the DZ correlation ($r_{MZ} < 2r_{DZ}$), there is evidence for common environmental effects. If there is no MZ and DZ correlation, there is evidence for unique environment effects only. The magnitudes of these correlations reflect the size of the relationship between the underlying factors.

In the full ACE univariate model, MZ and DZ twins are assumed to have different degrees of correlation for the genetic factors A but the same degree of correlation for the common environmental factors C. The correlations between genetic factors A will be 1 for MZ and 0.5 for DZ twins, whereas the correlations between common environmental factors C

will be 1 for both MZ and DZ twins. Unique environmental factors E are not correlated for MZ or DZ twin (Figure 2-1).

Figure 2-1 A path diagram for the basic ACE univariate twin model.

Observed variables for twin 1 and twin 2 are shown in rectangles. Latent (unmeasured) variables are shown in circles. A single-headed arrow indicates a direct influence of one variable on another (causal paths). A double-headed arrow between two variables indicates a correlation without any directional relationship. A indicates additive genetic factors; C shared environmental factors; E is unique environmental factors and a , c , e are path coefficients for A, C and E factors respectively. The correlations between i) additive genetic factors A are set at 1 for MZ and 0.5 for DZ twins and ii) common environmental factors C are set at 1 for both MZ and DZ twins.



Path analysis can be used to derive predicted variance and covariance for twin models. For the basic univariate twin model (Figure 2-1), the variance of the phenotype is the sum of the square of all paths pointing to it [$a^2 + c^2 + e^2$]. The covariance between twin 1 and 2 is the

product of the paths linking the phenotype via A, C and E respectively. Therefore, the genetic covariance for MZ twins is $[a * 1 * a] = a^2$ and for DZ twins, it is $[a * 0.5 * a] = 0.5a^2$, whereas the covariances due to C is c^2 for both MZ and DZ twins respectively. E does not contribute to the covariances between twins. Given the total covariance between two traits is the sums of all the paths connecting them via A and C, the expected variances and covariances of the traits within MZ and DZ twins can then be written as:

Cov for MZ =

$$\begin{bmatrix} \sigma_A^2 + \sigma_C^2 + \sigma_E^2 & \sigma_A^2 + \sigma_C^2 \\ \sigma_A^2 + \sigma_C^2 & \sigma_A^2 + \sigma_C^2 + \sigma_E^2 \end{bmatrix}$$

Cov for DZ =

$$\begin{bmatrix} \sigma_A^2 + \sigma_C^2 + \sigma_E^2 & 0.5\sigma_A^2 + \sigma_C^2 \\ 0.5\sigma_A^2 + \sigma_C^2 & \sigma_A^2 + \sigma_C^2 + \sigma_E^2 \end{bmatrix}$$

The Falconer's formula (Falconer & Mackay, 1996) can be used to estimate all standardized sources of variance. Broad-sense heritability (h^2), c^2 and e^2 are estimated by exploiting the difference in correlation between MZ (r_{MZ}) and DZ (r_{DZ}) twins in the following way:

$$h^2 = 2 (r_{MZ} - r_{DZ})$$

$$c^2 = 2r_{DZ} - r_{MZ} \quad \text{or} \quad r_{MZ} - h^2$$

$$e^2 = 1 - h^2 - c^2$$

2.5 Bivariate Genetic Analysis

The research question for this project is whether there is a genetic overlap between T2DM and depression. We therefore extend the model-fitting approach to bivariate genetic analysis. The observed correlation matrix is extended to a 4x4 matrix for each of the MZ and DZ twin groups (Figure 2-2) Sections b and d are the within-trait, cross-twin correlations for each trait. We inspect the MZ:DZ ratio of each separately to infer the mode of inheritance, as discussed in the “univariate genetic analysis”

section. Section a is the correlation between the two traits, also known as phenotypic correlation or cross-trait, within-twin correlation. If this correlation is significant, it suggests common aetiology influences (Rijsdijk & Sham, 2002). To determine whether common aetiology is due to genetic or common environmental factors, we examine the MZ:DZ ratio of the cross-trait, cross-twin correlations (section c in Figure 2-2). If the MZ:DZ correlation ratio is 2:1, it indicates that additive genetic effects drive the association between the traits, whereas a 1:1 ratio suggests common environmental effects drive the correlation between the trait. If the cross-trait, cross-twin correlations are non-significant, unique environmental effects are likely to be the predominant cause for the observed phenotypic correlation (or comorbidity).

Figure 2-2 Logic of bivariate twin analysis for T2DM and depression.

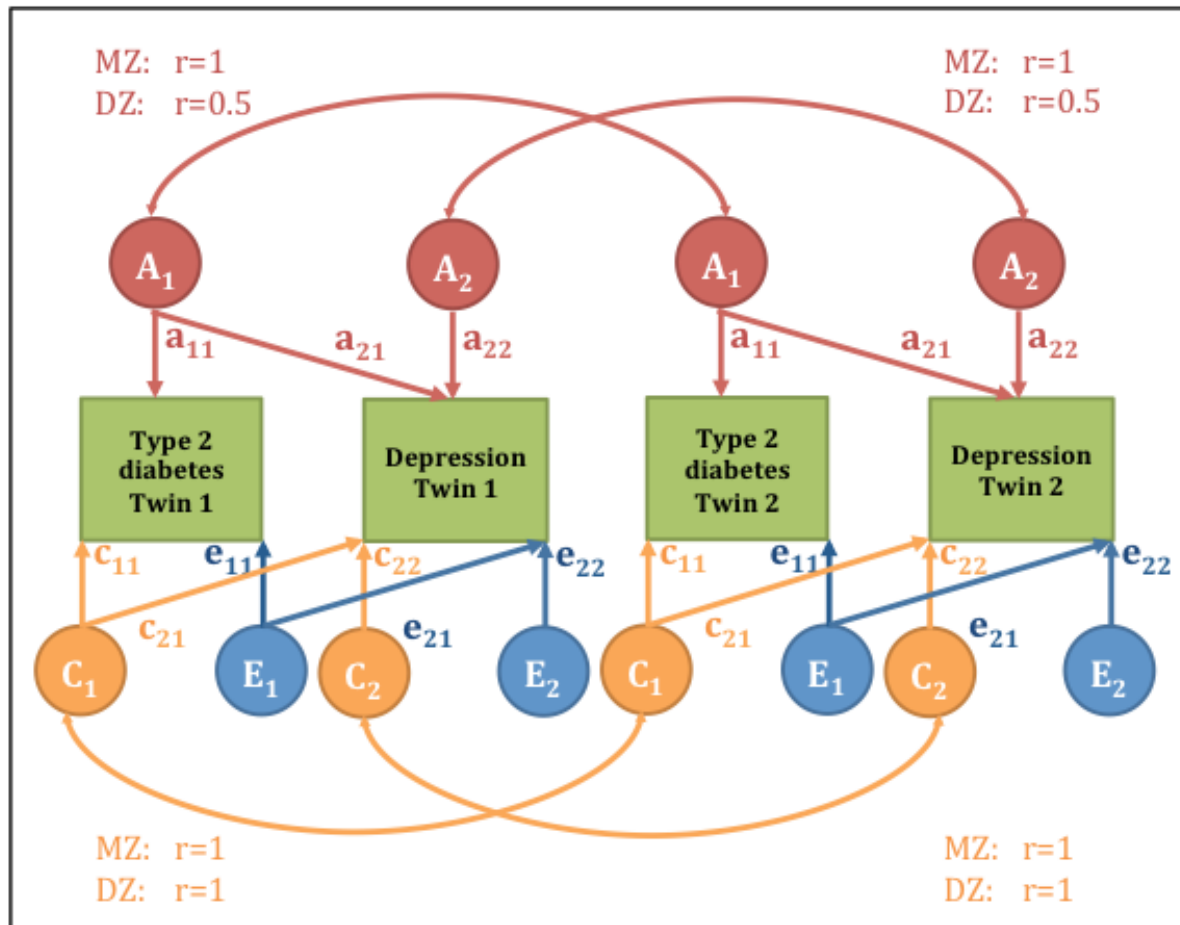
^aCross-trait, within-twin or phenotypic correlation between T2DM and depression.

^bWithin-trait, cross-twin for T2DM. ^cCross-trait, cross-twin between T2DM and depression. ^dWithin-trait, cross-twin for depression.

		Twin 1		Twin 2	
		T2DM	Depression	T2DM	Depression
Twin 1	T2DM	1			
	Depression	Cross-trait, within-twin ^a	1		
Twin 2	T2DM	Within-trait, cross-twin ^b	Cross-trait, cross-twin ^c	1	
	Depression	Cross-trait, cross-twin ^c	Within-trait, cross twin ^d	Cross-trait, within-twin ^a	1

The bivariate twin model can partition and estimate variances of T2DM and depression into genetic, common environmental and unique environmental factors (A, C and E respectively; Figure 2-3). Path analysis can again be used to derive predicted variance and covariance, since the covariance between twin 1 and 2 is the product of the paths linking the phenotypes via A, C and E respectively. Therefore, the genetic covariance for T2DM is $[a_{11} * 1 * a_{11}] = a_{11}^2$ in MZ twins and $[a_{11} * 0.5 * a_{11}] = 0.5a_{11}^2$ in DZ twins. For depression, it is $[a_{21} * 1 * a_{21}] + [a_{22} * 1 * a_{22}] = a_{21}^2 + a_{22}^2$ in MZ twins and $[a_{21} * 0.5 * a_{21}] + [a_{22} * 0.5 * a_{22}] = 0.5a_{21}^2 + 0.5a_{22}^2$ in DZ twins. For T2DM-depression, it is $[a_{21} * 1 * a_{22}] = a_{21}a_{22}$ in MZ twins and $[a_{21} * 0.5 * a_{22}] = 0.5a_{21}a_{22}$ in DZ twins. The paths for C and E follow the same logic. The bivariate Cholesky ACE model is depicted in Figure 2-3 and the expected variances and covariances of T2DM and depression within MZ and DZ twins summarised in the table below Figure 2-3.

Figure 2-3 Bivariate Cholesky ACE model and expected covariance of T2DM and depression within MZ and DZ twins.



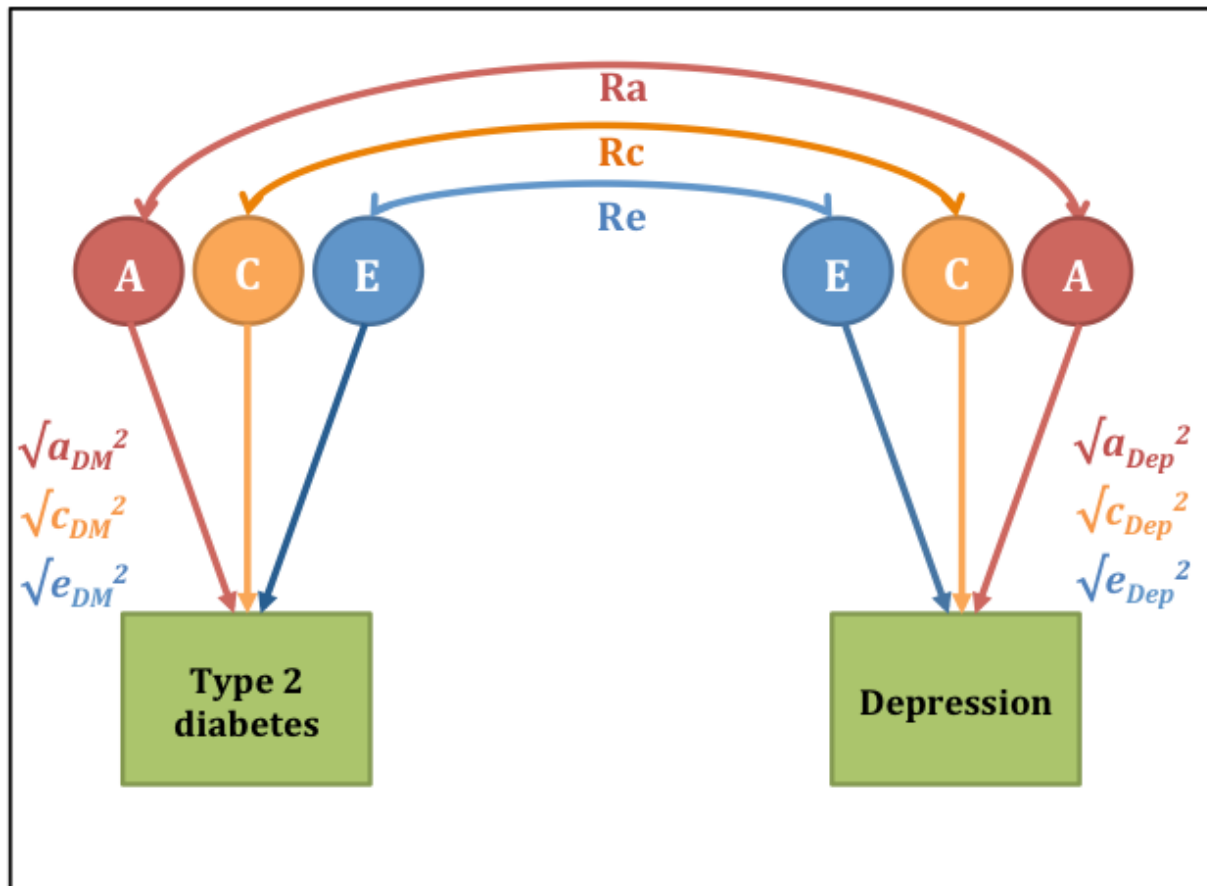
Observed T2DM and depression shown in rectangles. Latent variables are shown in circles. A double-headed arrow between two variables indicates a correlation without any directional relationship. A indicates additive genetic factors; C for shared environmental factors and E for unique environmental factors. a_{11}, c_{11} and e_{11} indicate path coefficients for A, C and E factors for T2DM, a_{22}, c_{22} and e_{22} for depression, and a_{21}, c_{21} and e_{21} for effect of T2DM on depression respectively. The correlations between i) additive genetic factors A are set at 1 for MZ and 0.5 for DZ twins and ii) common environmental factors C are set at 1 for both MZ and DZ twins.

MZ twins		Twin 1		Twin 2	
		T2DM	Depression	T2DM	Depression
Twin 1	T2DM	$a_{11}^2 + c_{11}^2 + e_{11}^2$		$a_{11}^2 + c_{11}^2$	
	Depression	$a_{11}a_{21} + c_{11}c_{21} + e_{11}e_{21}$	$a_{21}^2 + a_{22}^2 + c_{21}^2 + c_{22}^2 + e_{21}^2 + e_{22}^2$	$a_{11}a_{21} + c_{11}c_{21}$	$a_{21}^2 + a_{22}^2 + c_{21}^2 + c_{22}^2$
Twin 2	T2DM	$a_{11}^2 + c_{11}^2$		$a_{11}^2 + c_{11}^2 + e_{11}^2$	
	Depression	$a_{11}a_{21} + c_{11}c_{21}$	$a_{21}^2 + a_{22}^2 + c_{21}^2 + c_{22}^2$	$a_{11}a_{21} + c_{11}c_{21} + e_{11}e_{21}$	$a_{21}^2 + a_{22}^2 + c_{21}^2 + c_{22}^2 + e_{21}^2 + e_{22}^2$
DZ twins		Twin 1		Twin 2	
		T2DM	Depression	T2DM	Depression
Twin 1	T2DM	$a_{11}^2 + c_{11}^2 + e_{11}^2$		$0.5a_{11}^2 + c_{11}^2$	
	Depression	$a_{11}a_{21} + c_{11}c_{21} + e_{11}e_{21}$	$a_{21}^2 + a_{22}^2 + c_{21}^2 + c_{22}^2 + e_{21}^2 + e_{22}^2$	$0.5a_{11}a_{21} + c_{11}c_{21}$	$0.5a_{21}^2 + 0.5a_{22}^2 + c_{21}^2 + c_{22}^2$
Twin 2	T2DM	$0.5a_{11}^2 + c_{11}^2$		$a_{11}^2 + c_{11}^2 + e_{11}^2$	
	Depression	$0.5a_{11}a_{21} + c_{11}c_{21}$	$0.5a_{21}^2 + 0.5a_{22}^2 + c_{21}^2 + c_{22}^2$	$a_{11}a_{21} + c_{11}c_{21} + e_{11}e_{21}$	$a_{21}^2 + a_{22}^2 + c_{21}^2 + c_{22}^2 + e_{21}^2 + e_{22}^2$

The expected variances and covariances of T2DM and depression can be standardised to generate standardised variance components on the paths and correlations between the latent variables for A, C and E factors (a_{DM} , c_{DM} , e_{DM} , a_{Dep} , c_{Dep} , e_{Dep} , Ra , Rc and Re respectively). The standardized estimates are then represented as a correlated factors model (Figure 2-4). The phenotypic correlation (Rph) for T2DM and depression can then be calculated as follow:

$$\begin{aligned} \mathbf{Rph} = & \left[\left(\sqrt{a_{DM}^2} \right) * Ra * \left(\sqrt{a_{Dep}^2} \right) \right] \\ & + \left[\left(\sqrt{c_{DM}^2} \right) * Rc * \left(\sqrt{c_{Dep}^2} \right) \right] \\ & + \left[\left(\sqrt{e_{DM}^2} \right) * Re * \left(\sqrt{e_{Dep}^2} \right) \right] \end{aligned}$$

Figure 2-4 Bivariate correlated factors model.



Only one twin from each pair is shown. Observed phenotype T2DM and depression are shown in rectangles. Latent variables are shown in circles. A indicates additive genetic factors; C for shared environmental factors and E for unique environmental factors. a_{DM} , c_{DM} and e_{DM} indicate path coefficients for A, C and E factors for T2DM and a_{Dep} , c_{Dep} and e_{Dep} for depression respectively. Ra , Rc and Re indicate correlations between the latent variables for A, C and E factors respectively.

2.6 Modelling sex difference

Sex is an important moderator for genetic and environmental effects. The classical twin design can be extended to accommodate the effect of sex on the variance decomposition by incorporating data from the following twin groups: i) same sex pairs (MZ-males, DZ-males, MZ-females, DZ-females) and ii) DZ-opposite sex pairs. The use of five twin groups allows both qualitative and quantitative sex differences in genetic and environmental effects to be examined. In “quantitative sex differences”, the same factors affect both males and females but sex modulates the magnitude of their effect, with their impact on the phenotypes being greater in one sex than the other. This will be characterised by different MZ:DZ ratios in male and female same-sex twins in the observed data. In “qualitative sex differences”, different factors are involved in males and females. This will be characterised by the DZ opposite-sex twin correlation being much smaller than the DZ same-sex correlation in the observed data.

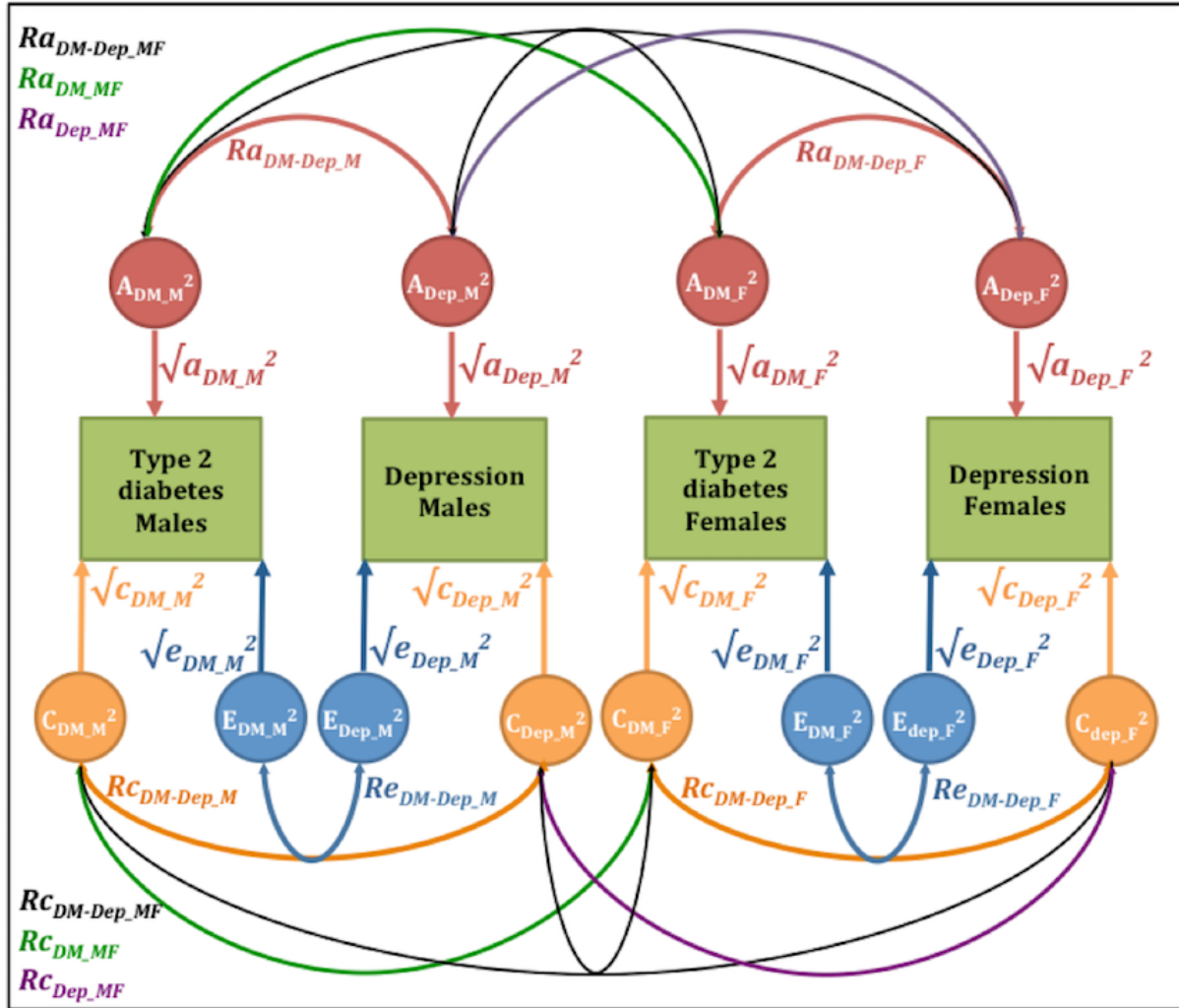
In “quantitative sex differences”, the genetic factors for DZ-opposite sex pairs are assumed to correlate in the same way as the DZ-same sex pairs. Thus, if a_m is the regression coefficient of a phenotype on the standardised genotype for males in a univariate sex-limitation model and a_f for females, then the genetic covariance will be $0.5a_m^2$ for DZ-males pairs, $0.5a_f^2$ for DZ-females pairs and $0.5a_ma_f$ for DZ-opposite sex twins. This assumption implies that the same set of genes influence the trait in both males and females (M.C. Neale & Cardon, 1992), but to a different degree as the magnitude of a_m can be different to a_f . The same principles apply to the common and unique environmental factors, with c_m differing from c_f and e_m from e_f .

In “qualitative sex differences”, however, there are expected sex-specific genetic and/or environmental influences to a trait, indicated by the smaller (sometimes negative) DZ opposite-sex correlation. To estimate this

effect, the correlation between the additive genetic factors in males and females (R_{AMF}) in opposite sex twin pairs is freely estimated to determine how different it is from the expected 0.5 as in the DZ same-sex pairs. Thus, if R_{AMF} is substantially different from 0.5, it suggests that different genetic factors are involved for that trait in males and females, indicating qualitative sex differences.

In bivariate model whereby sex-limitation modelling includes opposite-sex pairs, the Cholesky specification, as depicted in Figure 2-3, could cause problems. For example, the overall fit of the model may be dependent on the ordering of the variables if there are different modes of inheritance for the two traits across the sex (M. C. Neale, Roysamb, & Jacobson, 2006). The correlation approach is used to circumvent this problem by specifying explicit correlational paths between the A, C and E factors of the two traits in the model. In the “quantitative sex differences” model, the correlations between the additive genetic and common environmental factors for T2DM and depression across the male and females (R_{ADM_MF} ; R_{ADep_MF} ; $R_{ADM_Dep_MF}$; R_{CDM_MF} ; R_{CDep_MF} ; $R_{CDM_Dep_MF}$) are constrained to 0.5 and 1 respectively in the DZ-opposite sex pairs, as in the same-sex DZ twin pairs. In the “qualitative sex differences” model, R_{ADM_MF} , R_{ADep_MF} , $R_{ADM_Dep_MF}$, R_{CDM_MF} , R_{CDep_MF} and $R_{CDM_Dep_MF}$ are estimated as free parameters (Figure 2-5).

Figure 2-5 A general sex-limitation ACE model depicted for DZ-opposite sex twin pairs.



Subscript *M* indicates males; subscript *F* for females; Subscript *DM* indicates T2DM; subscript *Dep* for depression; *A* for additive genetic factors; *C* for shared environmental factors and *E* for unique environmental factors. Path coefficients for *A*, *C* and *E* factors are a_{DM_M} , c_{DM_M} , e_{DM_M} for T2DM and a_{Dep_M} , c_{Dep_M} , e_{Dep_M} for depression in males and a_{DM_F} , c_{DM_F} , e_{DM_F} , a_{Dep_F} , c_{Dep_F} and e_{Dep_F} in females respectively. Correlations between i) additive genetic factors are Ra ; ii) common environmental factors are Rc and iii) unique environmental factors are Re . In quantitative sex difference model, Ra_{DM_MF} , Ra_{Dep_MF} and $Ra_{DM_Dep_MF}$ are constrained to 0.5 and Rc_{DM_MF} , Rc_{Dep_MF} and $Rc_{DM_Dep_MF}$ to 1. In qualitative sex difference model, they are estimated as free parameters.

2.7 Liability threshold model

The primary variables of interest for this study are the presence/absence of clinically diagnosed/self-reported T2DM and depression. Because they are binary variables, a liability threshold model was used in the structural equation modelling. The model assumes that multifactorial diseases result from an underlying continuous character that is normally distributed in the population (Falconer, 1960). An individual will only be affected when his/her position on the liability distribution crosses a certain threshold. In the univariate twin model, there is a two-category ordered variable for each twin, namely affected or unaffected status in twin 1 and twin 2. The joint distribution of liabilities of twin pairs is assumed to follow a bivariate normal distribution, with a mean of 0, standard deviation of 1 and correlation unknown (Rijsdijk & Sham, 2002). These assumptions allow the relative proportion of individuals being affected or unaffected within each twin pair to be used to estimate the thresholds and correlation between the liabilities. Variance decomposition can be applied to liability, with correlation in liability being determined by path analysis.

2.8 Structural Equation Modelling

Structural equation modelling combines both variance components models and path analysis. It is the method most commonly used to analyse twin data. For this project, it was performed in the statistical software packages OpenMx (Verweij, Mosing, Zietsch, & Medland, 2012) in R. It fits the expectations of a model to the observed variances and co-variances of the (raw) data and produces the most likely estimates for the model by minimizing the differences via a fit function, such as maximum likelihood (M.C. Neale & Cardon, 1992). Raw maximum-likelihood allows us to handle data that are missing at random (data missingness) in order to derive unbiased estimates.

Two criteria are used to choose the best-fitting, parsimonious model: i) differences in minus twice the log-likelihood ($-2LL$) yielding a statistic equivalent to a χ^2 -test, with degrees of freedom equal to the difference in the numbers of parameters and ii) Akaike's Information Criterion (AIC) with lower values indicating a better balance between explanatory power and parsimony (Akaike, 1987). A difference in AIC of at least 10 indicates substantial support in favour of the more parsimonious model (Burnham & Anderson, 2002).

2.9 Limitation of the classic twin design

There has been ongoing debate in regard to the assumption that MZ twins share 100% of their genes in common while DZ twins share on average 50% of their genes. Whole genome sequencing efforts has provided evidences that DNA sequences difference between MZ twins are not large, while studies reporting the proportion of identity by descent sharing (the true amount of genetic material that DZ twins have inherited from the same parents) ranges between 42-58%, averaging at 50% (van Dongen, Slagboom, Draisma, Martin, & Boomsma, 2012; Visscher et al., 2007).

The equal environment assumption has faced more challenges. It has been suggested that MZ twins might share more similar post-natal environment compared to DZ twins. A study using mistaken diagnosis of zygosity has, however, suggested that the more similar treatment received by MZ twins is not due to their greater phenotypic similarity but as a result of their genetic identity, with more similar response being elicited from the environment (Kendler, Neale, Kessler, Heath, & Eaves, 1993; Martin et al., 1997). Using MZ twins separate at birth and reared apart can potentially address this criticism, but MZ twins who are reared apart are rare. In addition, studies have suggested that pre-natal developmental processes might influence the epigenetic resemblance of twins, with MZ twins being epigenetically more similar than DZ twins owing to non-genetic causes (van Dongen et al., 2012). This, in turn, will result in higher

MZ correlation relative to DZ correlation, overestimating the heritability of phenotypes that are epigenetically regulated. Further research will clarify this hypothesis.

There have also been concerns that the circumstances of gestation are significantly different between twins and singletons, in particular for MZ twins (Martin et al., 1997). This can, in turn, potentially lead to certain medical disorders being more common in MZ twins, both at birth and later in life (Phillips, 1993). Studies have found that twins, especially MZ twins, experience growth retardation in utero, but such disadvantages disappear by the age of 6, with similar mortality among twins and in the general population after the age of 6 (Christensen, Vaupel, Holm, & Yashin, 1995).

Another challenge to the classic twin design is that heritability may be inflated in twin studies due to suboptimal modelling of the common environment component and the effect of non-assortative mating (Golan, Lander, & Rosset, 2014). Genome-wide Complex Trait Analysis (GCTA) using data from genome-wide association studies (GWAS) of unrelated individuals provides an alternative approach for heritability estimates to be estimated (Yang, Lee, Goddard, & Visscher, 2011). Heritability estimates from GCTA are generally half when compared to twin studies (Plomin & Simpson, 2013). The discrepancy can in part be due to GWAS only capturing effects of SNPs with a minor allele frequency of greater than 1%. In addition, GCTA does not include non-additive interactions, such as gene-gene or gene-environment.

2.10 Conclusion

The classic twin design provides is a powerful tool for studying biological discordance in individuals with equivalent genetic background. The inclusion of DZ opposite-sex pairs allows the effect of sex to be examined. Population based twin registries are highly valuable resources,

with many being involved in large GWAS consortia, making an important contribution towards understanding the genetic architecture

Chapter 3

Genetic overlap between T2DM and depression in Swedish and Danish twin registries

“Sorrow compressed my heart, and I felt I would die, and then ... Well, then I woke up.”

~ Fyodor Dostoyevsky, The Dream of a Ridiculous Man

ORIGINAL ARTICLE

Genetic overlap between type 2 diabetes and depression in Swedish and Danish twin registries

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A bidirectional association between type 2 diabetes (T2DM) and depression has been consistently reported. Depression is associated with worse biomedical outcomes and increased mortality. The mechanisms underlying the association of T2DM with depression remain unclear. One possible question we can address is the extent to which the co-occurrence of diabetes and depression is due to correlated genetic and/or environmental risk factors. In this study, we performed structural equation model fitting to population-level data from the Swedish ($n=68\,606$) and Danish ($n=95\,403$) twin registries. The primary outcomes were clinical diagnosis of T2DM and depression using national hospital discharge registries. The phenotypic correlation between T2DM and depression is modest in both samples. In the Swedish sample, unique environmental effects explain a greater proportion of the covariance in males, whereas the association is primarily attributed to genetic effects in females. In the Danish sample, genetic effects account for the majority of the covariance in both males and females. Qualitative genetic sex differences are observed in both samples. We believe this is the first study to demonstrate significant genetic overlap between T2DM and depression.

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INTRODUCTION

Cross-sectional and longitudinal studies have consistently reported an association between type 2 diabetes (T2DM) and depression,^{1,2} with up to 60% increased risk for developing T2DM in individuals with depression and 15% increased risk for incident depression in those with T2DM.^{3,4} Depression has been associated with adverse effects on diabetes outcomes including suboptimal glycaemic control, complications and higher rates of mortality. Tentative evidence suggests a common biological pathway between T2DM and depression, as people with depression have increased levels of inflammation, hyperactivity in the hypothalamic-pituitary-adrenal axis and sympathetic nervous system.⁵ Those biological processes also underlie T2DM.⁶

A significant genetic correlation between T2DM and depression would provide evidence in support of a common genetic pathway to both disorders. Although it is known that genetic factors are aetiologically important in both T2DM and depression, it is unclear to what extent the T2DM-depression association correlated genetic or environmental factors. Twin studies provide a valuable approach to investigate genetic influences on complex traits or disorders,⁷ as well as the genetic architecture of comorbidity. They use the similarity within and between diseases across monozygotic (MZ) and dizygotic (DZ) twins to estimate the relative importance of the effects of genetic, shared and individual-specific environmental factors on the variance and covariance of the disorders.

Two twin studies have addressed the genetic comorbidity of T2DM and depression. The Vietnam Era Twin Study of Aging

reported a significant odd ratio between T2DM and depression (1.7 (95% confidence interval: 1.1–2.7)) in a sample restricted to males aged 50–59. They concluded that there was no significant genetic correlation (0.19 (0–0.46)) or unique environmental correlation (0.09 (0–0.45)) between T2DM and depression.⁸ A study based on the Screening Across the Lifespan Twin (SALT) study reported a significant phenotypic correlation (0.20 (0.09–0.33)) in combined analyses of male and female twins aged 40 years and older. They concluded that there was significant environmental correlation (0.54 (0.02–0.88)) between T2DM and depression.⁹

Although current evidence does not support a common genetic pathway hypothesis, a genetic model that incorporates possible quantitative and qualitative sex differences in genetic and environmental effects is called for, given the substantially higher prevalence rate of comorbid T2DM and depression in females compared with males.^{10–12} We therefore aimed to examine the genetic aetiology of the T2DM-depression association in two large population samples of twins using sex-limitation genetic modelling. In this study we combined the data from the Swedish and Danish twin registries, as they have a similar ethnic population structure.

MATERIALS AND METHODS

Sample

The Swedish Twin Registry (<http://ki.se/twinreg>) covers over 99% of all twins born in Sweden between 1886 and 2000 (~160 000 individuals).¹³ This study will include data from the following two cohorts: (i) SALT

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($n=44\,113$) and (ii) Swedish Twin Studies of Adults: Genes and Environment (STAGE; $n=24\,493$). The SALT cohort consists of twins born between 1886 and 1958,^{13,14} whereas the STAGE cohort consists of twins born between 1959 and 1985.¹⁵ The project was approved by the Regional Research Ethics Board at Karolinska Institute and the Steering Board of the Swedish Twin Registry.

The Danish Twins Registry covers twin births in Denmark since 1870, with 170 000 individuals registered as of January 2011.¹⁶ This study will include all birth cohorts (1870–1930, 1931–1952, 1952–1982 and 1983–2000), excluding participants aged < 18 at time of data retrieval. The project was approved by the Danish Data Protection Agency.

Primary variables from hospital registry records

The Swedish National Patient Registry has coverage of inpatient care for medical and psychiatric disorders since 1963 on a regional basis, extended to complete coverage in 1987 and further expanded to include outpatient care in 2001. Disorders were coded according to the International Classification of Disease (ICD) 8th, 9th or 10th Editions. The Swedish Twin Registry is linked to the National Patient Registry using a unique personal registration number assigned to all residents of Sweden. Data were extracted from 1969 to 31 December 2010.

The Danish National Patient Register initially had coverage of inpatient care for somatic disorders in 1977, and extended to complete coverage of all inpatient and outpatient care for medical and psychiatric disorders since 1995.¹⁷ Disorders were coded according to the ICD 8th or 10th Editions. The Danish Twin Registry is linked to the National Patient Registry using a unique personal identification number recorded in the Danish Civil Registration System. Data were extracted from 1977 to 1 April 2011.

The primary outcomes are ICD diagnoses of T2DM and depression from the respective National Hospital Discharge Registry, defined a priori to maximise statistical power. T2DM is defined by the following codes: (i) ICD-8: 250; (ii) ICD-9: 250.00, 250.02, 250.10, 250.12, 250.20, 250.22, 250.30, 250.32, 250.40, 250.42, 250.50, 250.52, 250.60, 250.62, 250.70, 250.72, 250.80, 250.82, 250.90, 250.92 and (iii) ICD-10: E11. Depression is defined by the following codes: (i) ICD-8: 296.0, 296.2, 296.9, 298.0, 300.4; (ii) ICD-9: 296.2, 296.3, 300.4, 301.10, 301.12, 301.13, 309.10, 311 and (iii) ICD-10: F32, F33, F34.1 and F38.1 (Supplementary Table 1).

Secondary variables

Zygosity of same-sex twin pairs was based on standard self-report items and, when validated with biological markers, has an accuracy of 95–99% in the Swedish Twin Registry¹⁸ and 96% in the Danish Twin Registry.¹⁹ Demographic details included age and sex. The demographic data were retrieved on 31 December 2010 for the Swedish twin sample and 11 October 2014 for the Danish twin sample.

Statistical analysis

Genetic model fitting. The classical twin method is based on comparison of MZ twins who share 100%, whereas DZ twins share on average 50% of segregating genes. In the univariate ACE model, individual differences in a trait are assumed to arise from: (i) additive genetic influences (A), (ii) shared environmental influences (C) and (iii) unique environmental influences (E). In bivariate twin analysis, in addition to the variance components of the individual traits, the main goal is to decompose the phenotypic correlation between them into parts due to correlating additive genetic (rA), shared environmental (rC) and unique environmental (rE) effects predisposing to both traits.⁷ Quantitative sex differences are modelled by specifying sex-specific ACE paths for T2DM and depression as well as rA, rC and rE.²⁰ The power is derived from different MZ-DZ correlation ratios across sex. Qualitative sex differences are incorporated by specifying free correlational paths between (i) all male and female genetic factors or (ii) all male and female common environmental factors in DZ opposite-sex twin pairs. The power is derived from differential within-trait and cross-trait correlations in opposite-sex compared with same-sex DZ pairs.²⁰

Genetic model fitting analysis was performed in the programme OpenMx.²¹ A liability threshold model was used, assuming that the risk to T2DM and depression is each normally distributed in the general population with the disorder manifesting when a certain threshold of risk is exceeded.²² Their joint distribution is assumed to have a bivariate normal distribution, with the relative proportions of concordant (both twins above or below the thresholds) and discordant pairs informing on the correlation between the liabilities. Given the range of birth year from

1896 to 1985 in the Swedish Twin Registry and from 1870 to 2000 in the Danish Twin Registry, age at the time when the demographic data were retrieved was modelled as a covariate on the thresholds.

Two criteria were used to choose the best-fitting, parsimonious model: (i) differences in minus twice the log-likelihood (–2LL) yielding a statistic equivalent to a χ^2 -test, with degrees of freedom equal to the difference in the numbers of parameters and (ii) Akaike's Information Criterion (AIC),²³ with lower values indicating a better balance between explanatory power and parsimony. A difference in AIC at least 10 indicates substantial support in favour of the more parsimonious model.

Secondary analysis. The genetic model fitting analysis was repeated and restricted to individuals born after 1950 in both the Swedish and Danish samples.

RESULTS

Descriptive finding

The Swedish Twin Registry from the SALT and STAGE cohorts consists of 68 606 twin individuals with known age, sex, zygosity, T2DM and depression. This results in 50 082 twin pairs and 18 524 single twins (Table 1). The mean age was 58.5 (17.6) as of 31 December 2010 when the demographic data were retrieved.

The Danish Twin Registry consists of 115 886 twin individuals, of whom 95 403 individuals have known age, sex, zygosity, T2DM and depression status and are aged over 18. This results in 94 194 twin pairs and 1209 single twins (Table 1). The mean age was 60.0 (16.8) as of 11 October 2014 when the demographic data were retrieved.

In both the Swedish and Danish samples, the prevalence rates for T2DM and depression were similar for males and females (Table 1). Mean-centred age was incorporated as a covariate, and thus, the thresholds and other estimates apply to a sample at mean age. To illustrate, the prevalence rate for T2DM in males for the Swedish sample is 4.7% (z-value=1.67) at age 59. With the effect of age being estimated at –0.03, T2DM prevalence rates will be 1.3% at age 40 (z-value=2.24) and 9.0% at age 70 (z-value=1.34).

Logistic regression analysis revealed a positive relationship between T2DM and depression in males and females in the Swedish (Rph: males 0.13 (0.08–0.14); females 0.16 (0.12–0.17) and Danish (Rph: males 0.16 (0.12–0.20); females 0.15 (0.12–0.20)) samples. Tetrachoric correlations stratified by zygosity and sex are summarised in Supplementary Table 2.

Genetic model fitting in the Swedish twin sample

The starting point was an ACE model that included both quantitative and qualitative sex differences in genetic factors (model 1S: –2LL=52 061.97; df=137 186; AIC=–222 310). The effect of common environment was negligible, shown by a non-significant decline in fit of the AE model (model 2S: –2LL=52 063.00; df=137 192; AIC=–222 321; $\chi^2=1.04$; $P=0.98$). To test the significance of qualitative sex differences, all genetic factors were constrained to correlate at 0.50 across males and females in opposite-sex pairs model, resulting in a significantly poorer fit of the AE model (model 3S: –2LL=52 116.61; df=137 196; AIC=–222 275; $\chi^2=53.61$; $P<0.05$). All A and E paths are therefore estimated freely for males and females with no further constraints, as it was not possible to equate them without a significant worsening of fit.

The best-fitting model is therefore the full sex-limitation bivariate AE model (Figure 1), with both quantitative and qualitative sex differences. The heritability estimates for clinical diagnoses of T2DM and depression were 66% (58–73%) and 45% (32–56%) in males and 71% (65–77%) and 38% (30–47%) in females, respectively. The genetic correlation between T2DM and depression was non-significant in males (0.06 (–0.13–0.25)) but

Table 1. Descriptive summary of MZ and DZ twins in Swedish and Danish twin samples, stratified by sex

Sample		Swedish		Danish	
		Males	Females	Males	Females
Number of paired twins	MZ	6524	9270	11 006	11 110
Number of single twins		2335	2131	59	59
	DZ	7440	9770	19 538	17 640
		3757	3421	130	113
	Opposite-sex	17 078		34 900	
		6880		848	
	Total	50 082		94 194	
		18 524		1209	
Age, mean (s.d.)	MZ	54.9 (17.9)	55.0 (18.7)	58.3 (16.8)	59.5 (18.3)
	DZ	60.3 (16.8)	61.3 (18.0)	60.5 (16.1)	63.0 (17.7)
	Opposite-sex	59.2 (16.5)		58.6 (15.7)	
	Total	58.5 (17.6)		60.0 (16.8)	
Prevalence of T2DM (%) ^a	MZ	5.5	4.2	4.1	3.3
	DZ	6.9	5.2	4.7	4.2
	Opposite-sex	5.8		3.1	
Prevalence of depression (%) ^a	MZ	3.7	5.8	2.8	4.7
	DZ	3.7	5.7	3.2	5.7
	Opposite-sex	4.9		3.8	
Proband wise concordance rate for T2DM (Number of concordant pair, discordant pair)	MZ	0.42 (69, 190)	0.43 (75, 195)	0.37 (83, 287)	0.33 (60, 247)
	DZ	0.20 (49, 398)	0.23 (53, 350)	0.14 (64, 764)	0.18 (66, 605)
	Opposite-sex	0.18 (91, 813)		0.13 (66, 886)	
Proband wise concordance rate for depression (Number of concordant pair, discordant pair)	MZ	0.21 (22, 174)	0.22 (57, 399)	0.19 (29, 243)	0.23 (59, 400)
	DZ	0.06 (8, 249)	0.05 (13, 489)	0.05 (16, 591)	0.16 (77, 837)
	Opposite-sex	0.09 (37, 706)		0.09 (61, 1170)	

Abbreviations: DZ, dizygotic twins; MZ, monozygotic twins; T2DM, type 2 diabetes. ^aIn the genetic modelling, the thresholds and other estimates apply to the sample at mean age. For example, with the effect of age being estimated at -0.03 for males in the Swedish Twin Registry, T2DM prevalence rates will be 1.3% at age 40 and 9.0% at age 70.

significant in females (0.23 (0.07–0.38)). In males, the phenotypic correlation between T2DM and depression was 31% due to genetic factors and 69% due to individual-specific environmental factors, whereas in females, these values were 75% and 25%, respectively.

Under the assumption of random mating, all correlations between genetic factors are expected to be 0.50 in same-sex DZ sibling pairs.²⁴ The estimated correlation between male and female genetic factors for T2DM ($A_{DM}M$ and $A_{DM}F$) was 0.38 (0.28–0.49) and for depression ($A_{DM}M$ and $A_{DM}F$) is 0.47 (0.25–0.50; Figure 1). They are close to the expected value of 0.50, suggesting little or no qualitative sex difference within diabetes and depression, respectively. The estimated correlations between the genetic factors for (i) male T2DM and female depression ($A_{DM}M$ and A_{DF}) and (ii) male depression and female T2DM ($A_{DM}F$ and A_{DF}) were -0.03 (-0.21 – 0.14) and 0.09 (0.07 – 0.10), respectively. They are substantially different from 0.50, indicating that different genetic factors are at play in males and females when it comes to explaining the T2DM–depression association (qualitative sex difference).

Genetic model fitting in the Danish twin sample

The same approach was adopted in the Danish sample. The starting model was the ACE model with both quantitative and qualitative sex differences in genetic factors (model ID: $-2LL = 58\,776.30$; $df = 176\,878$; $AIC = -294\,979.7$). The effect of common environment

was again negligible, as shown by a non-significantly decline in fit (model 2D: $-2LL = 58\,778.83$; $df = 176\,884$; $AIC = -294\,989.2$; $\chi^2 = 2.52$; $P = 0.87$). The effect of qualitative sex difference was important, as when all genetic factors were constrained to correlate at 0.50 across males and females in the opposite-sex pair model, a significantly poorer fit of the AE model resulted (model 3D: $-2LL = 58\,838.40$; $df = 176\,888$; $AIC = -294\,937.6$; $\chi^2 = 62.10$; $P < 0.05$). All A and E paths are therefore estimated freely for males and females with no further constraints.

The best-fitting model is also the full sex-limitation bivariate AE model (Figure 2), with both quantitative and qualitative sex differences. The heritability estimates for clinical diagnoses of T2DM and depression were 67% (61–73%) and 45% (35–54%) in males and 66% (59–72%) and 53% (46–59%) in females, respectively. In both males and females, the genetic correlation between T2DM and depression was moderate and significant (males: 0.25 (0.23–0.41); females: 0.18 (0.06–0.31)). The phenotypic correlation was primarily attributed to shared genetic effects in both sexes (males: 87%; females: 74%).

The estimated correlation between male and female genetic factors for T2DM ($A_{DM}M$ and $A_{DM}F$) was 0.42 (0.31–0.50) and for depression ($A_{DM}M$ and $A_{DM}F$) was 0.40 (0.26–0.50; Figure 1). They are close to the expected value of 0.50, suggesting little or no qualitative sex difference within diabetes and depression, respectively. The estimated correlations between the genetic factors for (i) male T2DM and female depression ($A_{DM}M$ and A_{DF}) and (ii) male depression and female T2DM ($A_{DM}F$ and A_{DF}) were

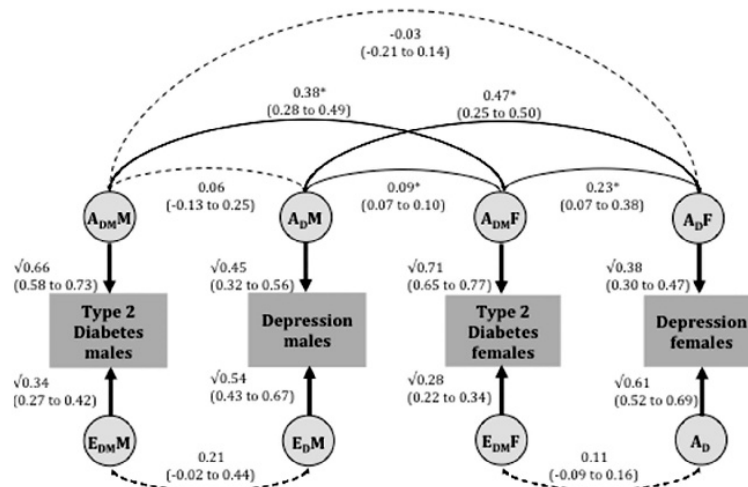


Figure 1. Parameters estimates from bivariate AE twin models for type 2 diabetes and depression, using the Swedish twin sample, with age as a covariate. Best-fit full sex-limitation bivariate AE model fit to Swedish data in opposite-sex dizygotic twins. Asterisk indicates a significant pathway. A indicates additive genetic effects; E for unique environmental effects; subscript $_{DM}$ for type 2 diabetes; subscript $_{D}$ for depression DM; M for males and F for females. Additive genetic and unique environment contributions to type 2 diabetes and depression are indicated by $A_{DM}M$, A_{DM} , $E_{DM}M$ and E_{DM} in males, and by $A_{DM}F$, A_{DF} , $E_{DM}F$ and E_{DF} in females, respectively.

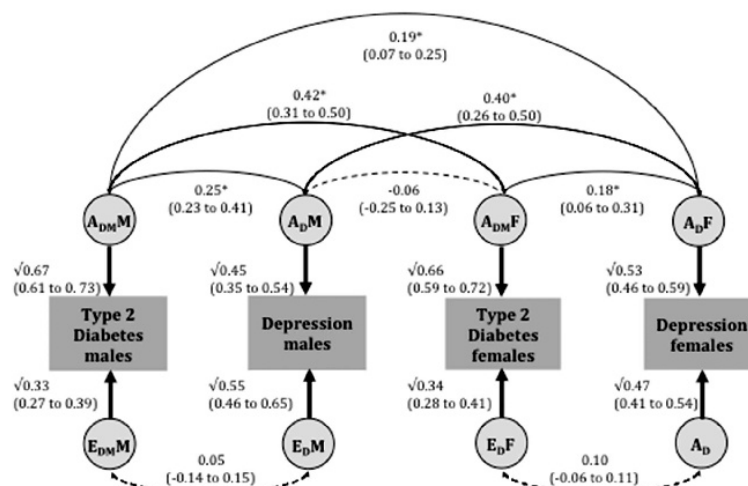


Figure 2. Parameters estimates from bivariate AE twin models for type 2 diabetes and depression, using the Danish twin sample, with age as a covariate. Best-fit full sex-limitation bivariate AE model fit to Danish data in opposite-sex dizygotic twins. Asterisk indicates a significant pathway. A indicates additive genetic effects; E for unique environmental effects; subscript $_{DM}$ for type 2 diabetes; subscript $_{D}$ for depression DM; M for males and F for females. Additive genetic and unique environment contributions to type 2 diabetes and depression are indicated by $A_{DM}M$, A_{DM} , $E_{DM}M$ and E_{DM} in males, and by $A_{DM}F$, A_{DF} , $E_{DM}F$ and E_{DF} in females, respectively.

0.19 (0.07–0.25) and -0.06 (-0.25 – 0.13), respectively. These are substantially different from 0.50, indicating that different genetic factors are involved in explaining the T2DM–depression association across sex (qualitative sex difference).

Secondary analysis

In samples restricted to individuals born after 1950, the best-fitting model is also the full sex-limitation bivariate AE model in both

the Swedish and Danish samples, with both quantitative and qualitative sex differences (data not shown). In the Swedish sample, the phenotypic correlation between T2DM and depression was significant in both males (0.21 (0.13–0.30)) and females (0.25 (0.17–0.33)). The heritability estimates for T2DM and depression were 73% (58–81%) and 52% (50–67%) in males and 72% (58–84%) and 40% (30–49%) in females, respectively. The genetic correlation between T2DM and depression was significant for females (males: 0.00 (-0.24 – 0.32); females: 0.49 (0.26–0.73)).

There is no major difference between the findings from the primary and secondary analysis.

In the Danish sample, the phenotypic correlation between T2DM and depression was significant in both males (0.11 (0.02–0.19)) and females (0.13 (0.04–0.22)). The heritability estimates for T2DM and depression were 78% (67–85%) and 45% (31–58%) in males and 78% (62–89%) and 47% (36–57%) in females, respectively. The genetic correlation between T2DM and depression was no longer significant (males: 0.07 (–0.18–0.32); females: 0.21 (–0.06–0.47)), in comparison with the primary analysis, as the 95% confidence intervals cross zero.

DISCUSSION

To the best of our knowledge, this is the first study to examine the genetic aetiology of the T2DM–depression comorbidity using hospital registry data from two large population samples of male and female twins covering the entire adult age range. The most notable feature of our finding is the similarity between the results from the Swedish and Danish samples. Both samples support the notion that qualitative genetic sex differences are at play in the genetic overlap between T2DM and depression. The main difference is the proportion of the T2DM–depression association due to shared genetic factors between Swedish and Danish samples. In the Swedish sample, the phenotypic relationship is mainly due to unique environment factors in males and shared genetic factors in females. In the Danish sample, it is due to shared genetic effects in both sexes. The observations were attenuated in the analyses restricted to individuals born after 1950. Although this could be a genuine difference, the 95% confidence intervals around the genetic correlations are overlapping between the primary and secondary analyses. In addition, the secondary analysis is half of the initial sample size for the Swedish sample (primary analysis: $n = 68\,606$; secondary analysis: $n = 35\,912$) and two fifths for the Danish sample (primary analysis: $n = 95\,403$; secondary analysis: $n = 40\,984$), leading to reduced power. The secondary analysis further strengthens the validity of our primary findings, as any observed cohort effect may potentially be secondary to multiple residual confounders. The findings, however, did not differ significantly between the entire sample and those born after 1950, especially in the Swedish sample.

Previous literature

Our results differ from previous findings. The Vietnam Era Twins Study of Aging⁸ suggests that there is no evidence of common genetic factors in contributing to the T2DM–depression association. The finding is difficult to interpret as it was restricted to males aged 50–59, relied on a much smaller sample and used self-reported data to construct the diagnosis of T2DM and depression.

The SALT study (using a cohort subsample included in our Swedish sample) previously reported a significant contribution of unique environmental factors in the T2DM–depression association, but insignificant contribution of genetic factors.⁹ Discrepancies in results could be due to reduced power, narrower age range and the use of computerised interview-based Composite International Diagnostic Inventory–Short Form instead of hospital registry data.

Implication

This is the first study examining genetic overlap in the T2DM–depression comorbidity in two large, population-based twin registries. Our finding of qualitative genetic sex differences implies that although the comorbidity is mainly due to correlated genetic risk factors, these factors are not necessarily the same in males and females. Possible biological support for this observed latent effect is given by findings regarding the activation

of the hypothalamic–pituitary adrenal axis, which has been postulated to have a major role in the T2DM and depression comorbidity.²⁵ A preliminary study reported sex difference between hypothalamic–pituitary adrenal axis activity and adrenergic polymorphisms in 189 patients with depression.²⁶ Increased hypothalamic–pituitary adrenal axis activity was associated with the ADRA2A genotype in males and ADRB2 genotype in females.

Other support for the observed qualitative sex differences in comorbidity is given by findings regarding visceral obesity, which has been suggested as a biological explanatory link between T2DM and depression comorbidity.^{25,27} Anthropometric traits such as waist-to-hip ratio are well-established proxy measures of abdominal fat distribution.^{28,29} Sex-specific dimorphic genetic effects have been reported for waist-to-hip ratios.³⁰ Of particular interest, a female-only association was reported for the peroxisome proliferator-activated receptors region.³¹ Proliferator-activated receptors- γ has a well-established role in T2DM treatment and has been implicated in its pathology.³² It has also been postulated as a potential target for the treatment of depression, as it is closely related to parainflammation and endoplasmic reticulum stress, processes that can potentially interfere with normal stress response in a depressive illness.^{33,34} Interestingly, the glucocorticoid receptor gene have been linked to both T2DM³⁵ and depression,³⁶ and its polymorphism has been associated with β -cell function in females, but not in males.³⁷

The findings from this study have potential clinical relevance. Our study demonstrates that the T2DM–depression comorbidity may be genetic in origin, with different genetic factors being at play in males and females. Future research would involve developing sex-specific biopsychosocial explanatory models and conducting sex-specific analysis in molecular genetic studies. Understanding the underlying mechanism of the T2DM and depression comorbidity will provide useful insight into identifying potential biological mechanisms that may be accessible to interventions.

Limitations

Our findings should be interpreted in the context of potentially significant limitations. No discrimination of types of diabetes was made in ICD-8 (1965) and the loss of participants at the time of retrieval of hospital registry data (2010 for the Swedish Twin Registry and 2011 for the Danish Twin Registry) may lead to misclassification of diabetes type. This is probably modest, given that T2DM comprises 90% of adults with diabetes.³⁸ Estimations obtained from a single database should, however, be interpreted with caution.³⁹ In addition, the mean age of onset for T2DM has been reported to be 46-year old⁴⁰ and we have included all individuals aged over 18 in both samples. It is therefore possible that individuals without a diagnosis of T2DM still have the propensity to develop the illness.

Another important limitation of our study is the use of national health registries. The Swedish and Danish National Hospital Discharge Registries do not include visits to general practitioners and have been shown to capture more severe cases.⁴¹ Our constructs of T2DM and depression might therefore capture a more severe spectrum of the disorders that warrant outpatient care and/or hospital admission. In addition, there is a small possibility of misclassifications of cases as controls, such as when a participant had a past history of depression before the inception of the registry and did not subsequently have further episodes of depression requiring outpatient or inpatient care.

Studies have also shown that males were less likely to seek help.⁴² The prevalence rates for diabetes and depression were similar across the sexes (Table 1). Anthropometric traits such as body mass index have not been adjusted for in this study and

we need to note the limitation that the observed genetic overlap can in part be due to the respective correlation of these traits with T2DM⁴³ and depression.⁴⁴ This and previous studies on T2DM and depression have focused on European-descent samples and replication in Asian and African descent populations are needed.

CONCLUSION

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Supplementary Information accompanies the paper on the Molecular Psychiatry website (<http://www.nature.com/mp>)

Chapter 4

Genetic overlap between T2DM and depression in a Sri Lankan population twin sample

“Given the choice between the experience of pain and nothing, I would choose pain.”

~ William Faulkner, The Wild Palms

Genetic overlap between T2DM and depression in a Sri Lankan population twin sample

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Abstract: 163

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Table: 2

Figure 1

Key words: T2DM, depression, genetic, twin, structural equation modelling, Sri-Lanka

4.1 Abstract

4.1.1 Background

A genetic overlap between type 2 diabetes (T2DM) and depression has been reported in two Scandinavian twin samples. This question has not been addressed in non-western populations. In this study, we aim to examine the aetiology of the T2DM-depression association in a Sri-Lankan population, using genetic model-fitting analysis.

4.1.2 Methods

The COTASS-2 consists of 2019 singletons, 842 monozygotic (MZ) and 1121 dizygotic (DZ) twin pairs. The primary outcomes were self-reported T2DM diagnosis and Beck Depression Inventory scores. Standard bivariate twin models were fitted to estimate the genetic and environmental covariance of the two traits.

4.1.3 Results

In the best-fitting model, the phenotypic correlation between T2DM and depression was significant in females only (0.15 (95% confidence interval (CI): 0.09-0.21)). This association was primarily attributed to a significant genetic correlation between the traits (0.52 (95% CI: 0.19-0.98)).

4.1.4 Conclusions

A significant genetic overlap between T2DM and depression in a non-western population was reported, in the context of a small phenotypic correlation.

4.2 Background

T2DM and depression are common disorders with considerable impact at personal, societal and national levels. An association between T2DM and depression is well documented in epidemiological studies, with up to 60% increased risk for developing T2DM in individuals with depression and 15% for incident depression in those with T2DM alone (Mezuk et al., 2008; Rotella & Mannucci, 2013). Depression is significantly associated with suboptimal glycaemic control, higher complication rates and increased mortality in people with T2DM (De Groot et al., 2001; Katon et al., 2005; Lustman et al., 2000). In addition, systematic inflammation, hypercortisolism and disturbed immune functions have been demonstrated to contribute to the T2DM-depression association (Kan et al., 2013; Laake et al., 2014; Renn et al., 2011). The underlying pathogenesis for comorbid T2DM and depression is likely to be complex, with both genetic and environmental factors playing a role. Genetic pleiotropy between T2DM and depression might explain some of the comorbidity observed.

Three twin studies have attempted to examine the genetic overlap between T2DM and depression. Two studies reported no evidence of correlated genetic factors (Mezuk et al., 2015; Scherrer et al., 2011), whereas one reported sex differences in the (genetic) association of T2DM and depression in two large Scandinavian populations (Kan et al., 2016). All previous twin studies were conducted in Western populations. Although the association between T2DM and depression has been observed in non-western populations (Arshad & Alvi, 2016; Hashim, Ariaratnam, Salleh, Said, & Sulaiman, 2016; Park et al., 2015), its genetic determinants have yet to be examined and there are reasons to suggest that these might be different. Compared to western populations, the prevalence of T2DM is rapidly increasing, with a younger age of onset and greater mortality (Ramachandran, Ma, & Snehalatha, 2010) whereas the prevalence of

depression is reported to be lower in non-western populations (Demyttenaere et al., 2004). In addition, previous twin studies have indicated that the genetic architecture of depression might be different in non-western populations, especially in males (Hur, 2008). In this study, we aimed to examine the genetic overlap of T2DM and depression in a South-Asian (Sri-Lankan) twin population sample using sex-limitation genetic model-fitting.

4.3 Methods

4.3.1 Sample

The Colombo Twin and Singleton Study (COTASS) is a population based sample of twins born in the Colombo district of Sri Lanka, with >90% participation rate (Siribaddana et al., 2008). COTASS-2 is a follow up of the original study and was conducted between 2012 and 2014, with >75% participation rate (Jayaweera et al., Under revision). In brief, COTASS-2 was designed to examine the relationship between mental health and metabolic risk factors. Demographic and phenotypic data were collected through extensive healthcare questionnaires whereas anthropometric and biological data were collected by trained research assistants.

4.3.2 Outcome variables

T2DM was defined as self-reported presence of medical diagnosis of T2DM. In addition, fasting blood glucose and HbA1c (glycated haemoglobin levels) were collected. Depression was measured using the Beck Depression Inventory (BDI) which captures depressive symptoms and severity in the past two weeks (Beck, Steer, & Brown, 1996). The BDI was translated into Sinhalese by a panel of clinical professionals fluent in both Sinhalese and English. The questionnaire was cross-culturally adapted in wording in order to best describe the questions in their meaning (Sumathipala & Murray, 2000). Secondary variables included self-reported age and sex. Zygosity of same-sex twin pairs was based on a standard self-

report questionnaire measure of similarity (Ooki, Yamada, & Asaka, 1993).

4.3.3 Statistical analysis

The classical twin method has three main assumptions: i) MZ twins share 100% and DZ twins share on average 50% of their segregating genes (additive genetic effects); ii) MZ and DZ twins are correlated for environmental influences to the same extent (equal environment assumption) and iii) mating in the population occurs at random (non-assortative mating). In a univariate ACE model, individual differences in a trait are assumed to arise from: additive genetic (A), common environmental (C) and unique environmental (E) influences. In a bivariate ACE model, in addition to the A, C and E components of each trait, the phenotypic correlation between two traits can be partitioned into correlating additive genetic (R_a), shared environmental (R_c) and unique environmental (R_e) effects (M.C. Neale & Cardon, 1992). Having same-sex male and female MZ and DZ twin pairs as well as opposite-sex twin pairs, allows testing for: i) “qualitative sex differences” where different genetic and common environmental factors are involved in males and females; and ii) “quantitative sex differences” where the same genetic and environmental factors are involved but the magnitude of their effect is modulated by sex. The power to estimate qualitative sex differences is based on differences of within-trait and cross-trait correlations in opposite-sex DZ pairs compared to same-sex DZ pairs, whereas the power to estimate quantitative sex differences is based on differential MZ and DZ within-trait and cross-trait correlations in same-sex twin pairs (M. C. Neale et al., 2006).

First, a full sex-limitation model was fitted in which the A, C and E parameters were allowed to differ between males and females. This tests for quantitative sex differences. In addition, for opposite-sex pairs, the correlations between the A factors and the C factors between males and females were estimated freely in succession. These two models were then

compared to the model in which the correlations between the A factors were constrained to 0.5 and those between the C factors to 1 in opposite-sex pairs, respectively. This allows us to test for qualitative sex differences. Equating the male and female parameters allows us to test for quantitative sex differences. The free and open source software programme OpenMx (Boker et al., 2011) was used for genetic model-fitting analysis on combined dichotomous T2DM data, requiring a liability threshold model (17), and continuous, log-transformed sex-and age-regressed BDI residual scores. Age effects on T2DM were modelled on the liability threshold (scripts available upon request).

Two criteria were used to choose the best fitting model: i) differences in minus twice the log-likelihood (-2LL) distributed as chi-square and ii) Akaike's Information Criterion (AIC), with lower values indicating a better balance between explanatory power and parsimony. A difference in AIC of ≥ 10 indicating support in favour of the more parsimonious model (Akaike, 1987; Burnham & Anderson, 2002).

4.4 Results

4.4.1 Descriptive Statistics

The CoTASS sample consisted of 3956 twin individuals (1963 twin pairs and 30 twin individuals) and 2019 singletons. The mean age was 43.0 (standard deviation (SD): 14.3) and BMI was 23.8 kg/m² (SD: 4.6; table 1). There were 471 cases of self-reported T2DM in total. For the entire sample, the mean fasting plasma glucose was 6.0 mmol/l (SD: 2.3). The mean HbA_{1c} was 42.1 mmol/mol (SD: 15.3) as per International Federation of Clinical Chemistry (IFCC) units and 6.0% (SD: 1.4) as per Diabetes Control and Complications Trial (DCTT) units. For individuals with a diagnosis of T2DM, the mean fasting plasma glucose was 8.9 mmol (SD: 4.3) and HbA_{1c} was 73.8 mmol/mol (SD: 47.0; DCTT: 8.9% (SD: 4.3)). For individuals who did not report a diagnosis of T2DM, the mean fasting

plasma glucose was 5.6 mmol/l (SD: 1.4) and HbA_{1c} was 38.8 mmol/mol (SD: 9.8; DCTT: 5.7% (SD: 0.9)).

The recommended diagnostic cut-off for T2DM using HbA_{1c} is IFCC: ≥ 48 mmol/mol (DCTT: 6.5%) and fasting plasma glucose is ≥ 7.0 mmol/l. Among individuals who self-reported T2DM and biological samples are available (n=426), 117 (27.4%) have HbA_{1c} and 164 (38.5%) have fasting plasma glucose below diagnostic cut-offs. Both HbA_{1c} and fasting plasmas glucose can be within the normal range among people with well-controlled T2DM and therefore, does not exclude T2DM being present. Among individuals who did not report to have a T2DM diagnosis and biological samples are available (n=2,967), 219 (7.4%) have HbA_{1c} and 140 (4.7%) have fasting plasma glucose below diagnostic cut-offs. This suggests a small proportion of individuals who fulfil the diagnostic criteria for T2DM but are unaware of the disease process in CoTaSS-2.

The mean BDI depression score was 4.9 (SD: 6.2), with 355 individuals scoring above 13, the cut-off for a clinical diagnosis of depression using the BDI. The BDI scores were positively skewed on visual inspection, with a kurtosis of 6.98 which reduced to -0.17 after log-transformation of the age and sex-regressed scores. In males, the phenotypic correlations between depression and i) T2DM, ii) fasting blood glucose and iii) HbA_{1c} were 0.06 (95% confidence interval: -0.02-0.14), 0.06 (0-0.11) and 0.06 (0.01-0.11) respectively. In females, they were 0.15 (0.09-0.21), 0.05 (0.01-0.10) and 0.06 (0.01-0.10). Correlations stratified by zygosity and sex are summarised in Table 4-2. Given the small phenotypic correlation between depression and both fasting blood glucose and HbA_{1c}, we focused the genetic model-fitting between depression and diagnosis of T2DM.

Table 4-1 Descriptive statistics of MZ and DZ twins, stratified by sex in COTASS-2.

		Males	Females
Number of paired twins	MZ	368	474
Number of single twins		4	4
	DZ	268	310
		7	14
	Opposite-sex	543	
		1	
Age, Mean (SD)	MZ	37.6 (12.5)	39.2 (12.8)
	DZ	39.3 (13.0)	42.9 (14.0)
	Opposite-sex	40.2 (13.2)	
Number of T2DM cases	MZ	40	51
	DZ	32	61
	Opposite-sex	82	
Beck Depression Inventory, Mean (SD)	MZ	3.7 (5.2)	4.6 (5.7)
	DZ	3.9 (5.5)	5.1 (6.1)
	Opposite-sex	4.6 (6.1)	
Proband wise concordance rate for T2DM (Number of concordant pair, number of discordant pair)	MZ	0.69	0.48
		11, 10	10, 22
	DZ	0.24	0.26
		3,19	6, 35
	Opposite-sex	0.25	
		8, 49	

Table 4-2 Correlations for i) T2DM, ii) depression and iii) T2DM–depression by zygosity and sex in COTASS-2.

		Correlation (95% CI)	
		Males	Females
Within Trait	Cross Twin		
T2DM	MZ	0.94 (0.82-0.98)	0.85 (0.70-0.94)
	DZ	0.44 (-0.03-0.76)	0.35 (0.01-0.62)
	Opposite-sex	0.44 (0.16-0.65)	
Depression	MZ	0.29 (0.15-0.41)	0.36 (0.25-0.45)
	DZ	0.24 (0.08-0.38)	0.22 (0.07-0.34)
	Opposite-sex	0.12 (0.01-0.22)	
Cross Trait	Cross Twin		
T2DM - Depression	MZ	0.12 (0-0.25)	0.21 (0.09-0.32)
	DZ	0.06 (-0.14-0.25)	0.06 (-0.09-0.21)
	Opposite-sex	-0.12 (-0.25-0.01)	

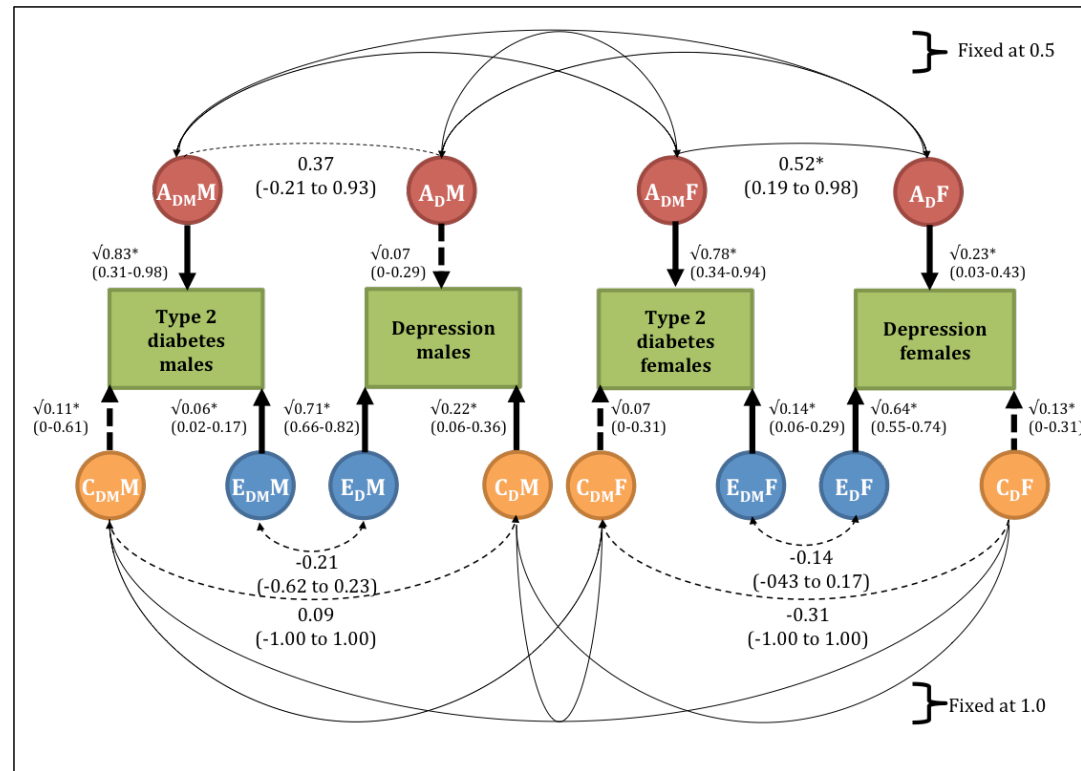
4.4.2 Genetic model-fitting

First, a sex-limitation ACE model including quantitative and qualitative genetic sex differences was fitted (HetACEg: $-2LL=15429.91$; $df=7799$; $AIC=-168.09$). Significance of qualitative genetic sex differences was tested by comparing this model to one in which the correlation between the A and C factors across males and females in opposite-sex pairs were constrained to correlate at 0.50 and 1 respectively, as is the case in same-sex DZ pairs (HetACE: $-2LL=15432.42$; $df=7803$; $AIC=-174.58$). This resulted in a non-significant decline in model-fit (HetACEg vs. HetACE: $\chi^2_{(df=4)}=1.52$; $p=0.82$), indicating that qualitative sex differences for the genetic factors were negligible. Secondly, a sex-limitation ACE model including quantitative and qualitative common environmental sex

differences was fitted (HetACEc: $-2LL=15431.33$; $df=7799$; $AIC=-166.67$). Compared to the HetACE model, this model also showed a non-significant decline in fit (HetACEc vs. HetACE: $\chi^2_{(df=4)}=0.097$; $p=1.00$). Thirdly, we tested for quantitative sex differences by equating the A, C and E parameters across males and females (HomoACE: $-2LL=15583.57$; $df=7812$; $AIC=-40.43$). Compared to the HetACE model, this resulted in a significant decline in fit (HomoACEc vs. HetACE: $\chi^2_{(df=9)}=152.15$; $p<0.0001$), indicating the importance of quantitative sex differences. The best-fitting model is therefore the sex-limitation model with quantitative sex differences (Figure 4-1).

Figure 4-1 Parameter estimates of the bivariate ACE twin model for T2DM and depression in COTASS-2.

Best-fit sex-limitation bivariate ACE model shown for opposite-sex DZ twin pairs. A indicates additive genetic factors, C common environmental factors, and E unique environmental factors; subscript $_{DM}$ indicates T2DM and $_D$ depression; M indicates males and F for females. Asterisks indicate a significant pathway.



Estimates of the standardized additive genetic, common and unique environment variance of the traits were different across sex for depression: 7% (95% CI: 0%-29%), 22% (95% CI: 6%-36%) and 71% (95% CI: 66%-82%) respectively in males, and 23% (95% CI: 3%-43%), 13% (95% CI: 0%-31%) and 64% (95% CI: 55%-74%) respectively in females. For T2DM the estimates were similar across sexes: 83% (95% CI: 31%-98%), 11% (95% CI: 0%-61%) and 6% (95% CI: 2%-17%) respectively in males, and 78% (95% CI: 34%-94%), 7% (95% CI: 0%-47%) and 14% (95% CI: 6%-29%) respectively in females. The genetic correlation between T2DM and depression was non-significant in males (0.37 (95% CI: -0.21-0.93) but significant in females (0.52 (95% CI: 0.19-0.98)). The significant phenotypic correlation in females is mainly due to correlated genetic factors.

4.5 Discussion

To our knowledge, this is the first study reporting a significant genetic overlap between T2DM and depression in females in a non-western population. Our findings in females are consistent with previous reports (Kan et al., 2016), with most of the phenotypic overlap ($r=0.15$; 95% CI: 0.09-0.21) observed being due to correlated genetic factors ($r_g=0.52$; 95% CI: 0.19-0.98). Although the magnitude of our genetic correlation is substantially higher, the wider confidence interval (0.52 (95% CI: 0.19-0.98)) overlaps with estimates derived from the Swedish (0.23 (95% CI: 0.07–0.38)) and Danish (0.18 (95% CI: 0.06–0.31)) twin samples.

The major differences between our findings and previous reports in Western populations are observed for males. Firstly, the phenotypic correlation was non-significant for males in our sample, whereas it is significant in both the Swedish (0.13 (95% CI: 0.08–0.14)) and Danish (0.16 (95% CI: 0.12–0.20)) twin samples. Secondly, our best-fitting model includes the effects of common environment, whereas previous twin studies do not (Mezuk et al., 2015; Scherrer et al., 2011). This might, in part, be explained by common environmental factors being more important

in explaining individual differences in depression for males in non-western populations like Sri-Lanka. The significant effects of common environment factors on depression have been previously reported in a Korean twin sample of adolescents and young adult males (32%;(Hur, 2008).

Our heritability estimates for depression were also significantly lower than those reported in a meta-analysis (~37%) (Sullivan et al., 2000). A possible explanation is that there is more room for environmental factors to explain individual differences in non-western populations, leading to a lower heritability estimates. Previous studies in the Sri-Lankan population have identified male-specific environmental factors to play a role in depression, namely unemployment, low levels of standard of living and living in more heavily urbanised areas (Ball et al., 2010). Our finding of significant common environmental effects in males might therefore reflect the differential economic and social pressures between the sexes in non-western populations and between western and non-western populations in general, and their subsequent effect on developing depression.

In addition to the explanation above, it is possible that a different phenotype of depression might be captured when a western instrument is used in a non-western population. A previous study using the Composite International Diagnostic Interview has suggested that the same underlying phenotype of depression was being studied, since the total number of depressive symptoms and pattern of symptoms endorsed were similar between the Sri-Lankan and Western populations (Ball et al., 2010). A study in the UK has reported that people of South Asian origin were more likely to disclose somatic rather than psychological symptoms when screening for non-psychotic psychiatric illness, although the extent of cultural variation in expressing psychological distress remains unclear and controversial (Hussain & Cochrane, 2004). Given that no specific measure of environmental factors was included in our analysis, we are

merely speculating on the nature and type of environmental factors that might contribute to the T2DM-depression association in non-western populations. If our findings are replicated in a larger non-western population twin sample, future studies could examine whether male-specific environmental factors modulate the aetiology of depression and its association with T2DM in non-western populations. Study specific differences also need to be considered in interpreting our findings. The COTASS-2 sample differs from previous twin studies in the assessment of T2DM and depression (self-report questionnaire/diagnosis verses hospital registry) and being a younger cohort.

4.5.1 Limitation

One major limitation of the study is the reliance on self-report questionnaires for assessing T2DM and depression. For T2DM, we explored the use of HbA_{1c} as a proxy marker, but the phenotypic correlation with depression was very small in magnitude. HbA_{1c} is a useful clinical biomarker for assessing glycaemic status and guiding treatment decisions for people with T2DM. It can, however, be within the normal range among people with well-controlled T2DM. For example, ~25% of individuals with T2DM in our study have a HbA_{1c} <48mmol/mol (6.5%), the recommended cut-off for diagnosing T2DM. HbA_{1c} alone might therefore not be a sufficiently reliable tool for recognising T2DM, especially during the early stages of the disease. In addition, being diagnosed with T2DM, initiating and implementing the associated diabetes self-management might have a greater impact on the development of depression than HbA_{1c} alone, explaining the differential phenotypic correlations between depression and i) T2DM diagnosis and ii) HbA_{1c}.

For depression, the BDI captures depressive symptoms for the past two weeks, and is not aimed to establish a diagnosis of major depressive disorder. Information about antidepressant was also not available at time of analysis, and thus, it is possible that individuals who were actively depressed and receiving antidepressant treatment were included in the

COTASS-2 sample. This can potentially affect their responses on the BDI. Psychiatric disorders remain under-recognised in Sri-Lanka. A scarcity of mental health resources and stigma have been identified as major barriers for communities to seek care (D'Souza & Singh, 2005). A recent national survey of self-reported health in Sri Lanka reported that only 23% of individuals reporting to have a mental illness receive any treatment (Ministry of National Policies and Economic Affairs, 2014). In addition, the self-report nature of a questionnaire can affect its results due to social desirability and respondent educational attainment (Cronbach, 1990). Thus, a more comprehensive approach would be to conduct structured diagnostic interview to screen for mental illnesses in the COTASS-2 sample, but it is both time and labour intensive.

Adopting a multi-informant approach, such as utilising a valid and reliable diagnostic interview for depression or cross-validating our measures with a clinical registry, could potentially strengthen our finding. Our study also utilises cross-sectional data, and thus, we cannot determine the extent by which individuals later develop T2DM or depression after being recruited into the study. A longitudinal design will allow us to examine changes in genetic and environmental influences in the clinical course of T2DM. Lastly, limitations of the classical twin model apply, namely the equal environment assumption and the assumption of negligible correlations between the A, C and E factors (Golan et al., 2014; van Dongen et al., 2012).

4.6 Conclusion

Our study strengthens previous reports of genetic factors playing an important role in the mechanism underlying the T2DM-depression link in females by replicating the finding in a non-western population and thus, demonstrating the generalizability of the finding. Two recent studies using a polygenic score approach in GWAS have, however, reported no evidence of a genetic overlap between T2DM and depression (Clarke et al.,

2016; Samaan et al., 2015). The reason for the discrepancy in findings between twin and GWAS studies is currently unclear and it appears that we have only begun to uncover the complex genetic underpinning of the T2DM-depression association.

4.7 Acknowledgments

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Chapter 5

Polygenic Score Analysis and LD Score Regression in Genome Wide Association Studies

“No truth can cure the sorrow we feel from losing a loved one. No truth, no sincerity, no strength, no kindness can cure that sorrow. All we can do is see it through to the end and learn something from it, but what we learn will be no help in facing the next sorrow that comes to us without warning.”

~ Haruki Murakami, Norwegian Wood

5.1 Overview

Findings from genome-wide association studies (GWAS) have opened up opportunities for estimating heritability from unrelated individuals, as an alternative to the traditional approaches of family and twin studies. With the increase in power of these large scale GWAS, it is becoming ever more informative to analyse the shared genetic aetiology between traits. This has led to growing evidence of shared and distinct genetic architectures among neuropsychiatric disorders (Han et al., 2016). Two methods that are used to investigate genetic overlap between disorders are polygenic score analysis and linkage disequilibrium (LD) score regression. Given that both T2DM and depression are considered to be polygenic disorders, with epidemiological studies reporting an association between them, we have applied both methods in this thesis to examine the polygenic component of these complex traits. In this chapter, I will focus on the statistics underling these methods. In brief, polygenic score analysis involves building polygenic risk scores for one phenotype and examining its association with another phenotype. Cross-trait LD score regression, on the other hand, uses information from LD and summary statistics from GWAS to estimate genetic correlation.

5.2 What is a GWAS?

A GWA is a method for hypothesis-free testing association between genetic variants and a trait using a sample of unrelated individuals. A GWAS may be a case control study, or a population cohort, with trait status known. It typically uses logistic regression to model the relationship between genotype and disease status to detect additive genetic effects. GWAS usually tests association between single nucleotide polymorphisms (SNP) and disease. SNPs are a common form of variation that span the genome. In GWAS, effect size is often reported as odds ratio (OR), a ratio between the odds of having a specific allele in diagnosed individuals (cases)

and the odds of having that same allele in healthy individuals (controls). Thus, if the odds ratio is greater than 1, the allele frequency is higher in cases than controls.

Technological advances have allowed SPNs to be efficiently and accurately genotyped using Illumina or Affymetrics genotyping arrays. These arrays predominantly capture common variation in the population, and can also be used to infer larger copy number variants. Given that about a million independent association tests are conducted to test common variation genome-wide, the multiple testing-correction threshold for a significant association is often set at 5×10^{-8} (0.05/1000000) (Chanock et al., 2007).

Because of the stringent multiple testing-correction threshold, large sample sizes are required to ensure that true association signals achieve sufficiently small p-values to reach genome-wide significance. Power has been a challenge for psychiatric genetic studies, especially in depression (Flint & Kendler, 2014). The power of a study is a function of its sample size, effect size, allele frequencies of risk loci, and population prevalence. The idea of an “inflection point” has been proposed, whereby the number of significant associations detected in a study increases approximately linearly with sample size once a critical minimum sample size is reached (Levinson et al., 2014). Below the inflection point, studies are underpowered to detect association with common SNPs, given small effect sizes. For schizophrenia, it is estimated that the inflection point is at 13000-18000 cases, with an estimate of 4 new GWA-significant SNPs per 1000 additional cases. Because of the higher prevalence and lower heritability of depression, the inflection point for depression was estimated at 75000-100000 cases (Levinson et al., 2014). The validity of this estimation was strengthened by a recent GWAS for depression based on self-reported data, which identified 15 genome-wide significant SNPs in a sample of 75607 cases and 231747 controls (Hyde et al., 2016).

Lack of statistical power as a result from small sample size can lead to type II error in conclusions drawn from GWAS. Statistical algorithms, such as the genomic-relatedness-based restricted maximum-likelihood implemented in Genome-wide Complex Trait Analysis (GCTA), have been developed to estimate the proportion of phenotypic variance explained by SNPs for complex traits. These estimates are thought to reflect the upper-bound of heritability directly derived from genotypes, if type II error is responsible for failure to find an association. These estimates were named “SNP-heritability” to distinguish them from the traditional estimates based on family data, and because they were extrapolated from the data based on genotyped platforms limited to common SNPs (Manolio et al., 2009; Yang et al., 2011). SNP-heritability are consistently lower than heritability estimates from twin studies, and the difference has been termed “missing heritability” (Manolio et al., 2009). Various theories have been proposed to explain the missing heritability, including rare variants (Zuk et al., 2014), epigenetics (Furrow, Christiansen, & Feldman, 2011), and gene-environment interactions (Kaprio, 2012). Advances in whole genome sequencing will identify rare variants and capture a greater proportion of causative common SNPs. This will reduce the magnitude of the “missing heritability” to some extent, but the effect of epigenetics and gene-environment interactions will remain unexamined and will need to be resolved.

5.3 Hardy-Weinberg equilibrium and LD

Hardy–Weinberg equilibrium states that allele and genotype frequencies are constant in a population across generations in the absence of evolutionary influences. Factors that can disrupt Hardy-Weinberg equilibrium include natural selection, non-random mating, genetic mutation, genetic drift and gene flow. For example, natural selection will favour the expression of alleles that bestowed survival advantage whereas non-random mating might lead to a greater expression of recessive

phenotypes. Both will result in changes in allele frequencies in a population. New alleles can be introduced into a population from *de novo* genetic mutations, or as a result of breeding between two populations (gene flow).

The various scenarios described above can lead to LD, a non-random association of alleles at two or more loci. If two loci are independently inherited, the haplotype frequencies are equal to the product of their corresponding allele frequencies in the population. The loci are in linkage equilibrium. If the two loci are not independently inherited, then the allele carried at one locus can be predicted from the allele at the second locus. LD is a measure of the deviation of observed haplotype frequency from its corresponding allelic frequencies expected under linkage equilibrium. LD therefore reflects the degree to which alleles at two loci are associated and is often standardised as the D' (relative measure of disequilibrium) or parameterised as the r^2 (squared Pearson coefficient of correlation) (Lewontin, 1988). One of the statistical method, described in this thesis and used to estimate trait and cross-trait heritability, is LD score regression, which exploits the relationship between LD and GWA test statistics.

5.4 Population stratification

Allele frequency can be systematically different between cases and controls due to ancestral differences, leading to spurious association between disease and the genetic variant (population stratification). This is of particular concern in large-scale association studies, as the effects of population stratification increases in proportion to the number of samples included. Large studies have sufficient power to identify very subtle differences in allele frequency between cases and controls that can be due to differences in genetic ancestry, and not due to differences in disease status (Reich & Goldstein, 2001). To control for confounding effect of population stratification, variables capturing these differences are

included in the logistic regression analysis as covariates. These variables are constructed from principal component analysis (PCA), a statistical method designed to account for variability in a dataset with large number of measurements by reducing the number of underlying dimensions to a few principal components (PCs). PCA has been therefore used in psychiatric genetics to identify differences in ancestry among populations and samples (Novembre et al., 2008). This allows us to address the problem of population stratification (Price et al., 2006) and in datasets with ancestral differences between samples, the PCs often capture geographic information.

5.5 Quality Control

Quality control is an essential part of a GWAS, since any error in assumptions can affect the distribution of the test-statistics at the extreme end while any artefact can bias the estimates. It is, however, a fine balance, since excessive and unnecessary quality control can lead to informative (non-random) missingness. The aims of post-genotyping quality control are therefore to minimise sample heterogeneity at an individual level and to minimise genotype-calling problem at SNP level. Using individuals from the same underlying genetic population for case-control ascertainment is essential to avoid introducing systematic biases. Subsequent assessment of data quality will allow identification of substandard markers or/and samples.

Some authors have advocated implementing quality control on a “per-individual” basis before on a “per-marker” basis, as any SNP that is removed from the dataset can potentially have an effect on the disease being studied (C. A. Anderson et al., 2010). Per-individual quality control of GWA data involves removing any duplicated or related individuals and identifying individuals with i) discordant sex classification and ii) missing genotype/heterozygosity rate which is an outlier. Identifying discordant sex-information informs us to the possibility of error in sample labelling.

Genotype information from the X-chromosome provides an easy way to check for any discordance with ascertained sex.

Handling SNP missingness is a particularly important step in ensuring quality control for GWAS datasets, as it is strongly related to SNP quality and can lead to both false positive and false negative association signals (Weale, 2010). False positive arises if the difference in the frequency of called genotypes between cases and controls is driven by effects other than the disease (type I error). In contrast, false negative occurs if we fail to gather enough statistical power to detect a true difference in allele frequency between case and controls (type II error). False positive is a more likely scenario if the cases and controls were collected and/or genotyped differently, whereas excessive missingness could result in both false positives and false negatives. A common threshold used for missing genotype rate is $<2\%$, whereby SNPs with a genotyping rate $\leq 98\%$ are automatically removed from further analysis.

SNP missingness is a particular challenge for low frequency variants, since genotyping quality tends to deteriorate with rarer variants. SNPs with low minor allele frequency (MAF) have less information available, making them more difficult to call with current genotype calling algorithms. To ensure quality control, SNPs with $MAF < 2\%$ are usually excluded (C. A. Anderson et al., 2010). The $MAF > 10/n$ threshold whereby n is the number of samples has also been suggested as a plausible threshold (Weale, 2010).

Another method for checking genotyping quality is to examine departure from Hardy-Weinberg equilibrium in controls. It is recommended that SNPs which severely deviate from Hardy-Weinberg equilibrium are carefully examined, instead of being excluded, since many genuine effects, including a strong signal of association, can lead to departure from Hardy-Weinberg equilibrium (Turner et al., 2011). Extreme departure from Hardy-Weinberg equilibrium is more likely to

result from failure of genotype calling and can be used to guide the quality control process (Wittke-Thompson, Pluzhnikov, & Cox, 2005).

5.6 Imputation

The main goal of imputation is to utilise LD patterns for extraction of further genomic information from reference population by predicting SNPs which were not genotyped. This, in effect, increases genotyping coverage, allowing individual studies using different genotyping platforms and populations to be combined for meta-analysis, thus increasing statistical power. Common SNPs can usually be imputed with high quality, since most SNPs are highly correlated with genotyped SNPs, but low frequency SNPs are poorly imputed.

5.7 Polygenic scores approach

Given that causal genetic variants identified in GWAS only explain a small amount of variation in a trait, it necessitated the introduction of polygenic scores approach. GWAS provides estimates of effect sizes, such as OR, for each SNP, but even for highly significant SNPs, the odds ratios are often < 1.05 . Each SNP therefore explains only a small component of the genetic architecture of the disease and is therefore of limited utility for prediction on its own. However, combining information on risk alleles across SNPs creates a continuous measure of genetic liability for a disease and provides a better predictor. Polygenic risk scores may be created from SNPs that reach genome-wide significance, or by combining information on SNPs which capture modest effects but do not reach genome-wide significance. Polygenic risk scores usually use SNPs that meet liberal p -value thresholds (P_T), such as 5×10^{-5} . A less stringent P_T increases the number of SNPs being included in the polygenic scores which, in turn, increases the ratio of false-to-true positive SNPs. The polygenic scores approach can, however, tolerate inclusion of some false positives, since the stringent genome-wide significance threshold commonly used (5×10^{-8}) can

lead to many causal variants being discarded (Wray et al., 2014). In addition, an increase in effect size of the polygenic score can also improve the statistical power of the study.

Two datasets are required to conduct a polygenic score analysis; i) a base dataset with GWA summary-level statistics, used to define the SNPs to be included in a polygenic risk scores, with their effect sizes as weights and ii) a target dataset with individual-level genotype data in which scores are constructed. The two datasets should be independent from each other. SNPs which are present in both base and target datasets are then identified. In the base dataset, SNPs in strong LD with other SNPs, typically based on a pairwise r^2 threshold of 0.1 across 250kb, are pruned, generating a list of independent SNPs with the strongest evidence of association within each LD region.

The clumped SNPs are then ranked by their association p-values. Subsets of clumped SNPs at various P_T , typically ranging from <0.001 to <0.5 , are generated (step 1 in Figure 5-1). The risk alleles and effect sizes, such as odds ratios (OR), for each SNP in the base dataset are added to generate polygenic scores for each individual in the target dataset (step 2 in Figure 5-1). A polygenic score is therefore the sum of the number of trait-associated alleles, weighted by $\log(\text{OR})$ estimated from the base dataset (S. M. Purcell et al., 2009):

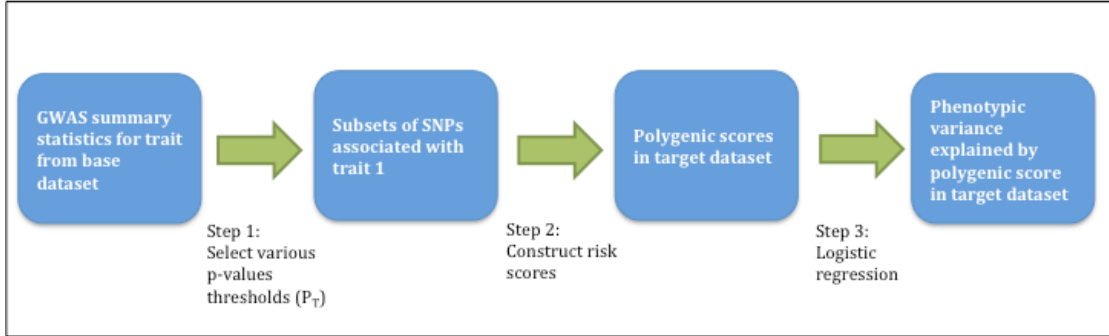
$$\text{Polygenic scores} = \sum_{i=1}^m \hat{\beta}_i G_i \quad [1]$$

where $\hat{\beta}_i$ is the effect size, such as $\log(\text{OR})$ and G_i is the number of risk alleles at the locus.

To test whether polygenic risk score predicts disease status in the target dataset, a logistic regression between polygenic scores and disease status is performed (step 3 in Figure 5-1). The proportion of phenotypic variance explained by a predictor for binary trait can be approximated by Nagelkerke's pseudo R-squared (R^2). For binary traits, such as disease case-control status, we compare the difference in the R^2 between the full

model (polygenic score and covariates) and the reduced model (covariates only) (Nagelkerke, 1991; S. M. Purcell et al., 2009; Wray et al., 2014). Covariates typically include genotyping platform, study sample and ancestry-informative PCs.

Figure 5-1 Polygenic score approach.



The polygenic score approach can be used to test whether the results from a GWAS base dataset predict the same trait in a target dataset. It may also be applied to cross-phenotype analysis which is the remit of our project. For example, we used the summary statistics from the stage 1 of Diabetes Genetics Replication And Meta-analysis Consortium (base dataset) (Morris et al., 2012) to generate T2DM-polygenic scores in deCODE (chapter 6). We then examined whether there is an association between T2DM-polygenic scores and the depression case/control status in deCODE using logistic regression.

5.8 LD score regression

The LD score regression is a statistical method that uses GWA summary-level results (B. K. Bulik-Sullivan et al., 2015). When applied to a bivariate model, it generates i) SNP-heritability for the two traits of interest ($h_{SNP_1}^2$ for trait 1 and $h_{SNP_2}^2$ for trait 2 respectively) and ii) SNP-correlation between the traits (r_{g_SNP}). It should be noted that $h_{SNP_1}^2$ and $h_{SNP_2}^2$ only captures additive heritability attributable to common genetic

variation (Lee, Wray, Goddard, & Visscher, 2011) and thus, reflecting the lower bound for the narrow-sense heritability generated from twin studies.

In LD score regression, LD score is estimated from a reference panel and is the sum of r^2 between a SNP and its surrounding SNPs. h_{SNP}^2 is then estimated by regressing the association test-statistics of a SNP from LD score. Genetic correlation is estimated by regressing the product of the Z-scores for SNP-trait association on the LD scores and standardising it by heritability estimates. For SNP variant j , the product of the Z-scores can be summarised as:

$$E[Z_{1j}Z_{2j}] = \frac{\sqrt{N_1N_2}\rho_g}{M} l_j + \frac{\rho N_s}{\sqrt{N_1N_2}} \quad [2]^1$$

where ρ_g is the genetic covariance, l_j is the LD score, N_1 is the sample size of study 1, N_2 is the sample size of study 2, M is the number of intersecting SNPs across both samples, N_s is the number of individuals in both samples and ρ is the phenotypic correlation among the overlapping samples. Z_{ij} is the Z-score test statistic from study i for association between trait and SNP j .

If study 1 and study 2 are the same study, then $N_1 = N_2 = N_s$, $\rho_g = h_g^2$ and $\rho = 1$. Equation [2] then reduces to the genetic covariance between a trait and itself which is the heritability, while the test-statistics become the product of the Z-scores ($\chi^2 = z^2$).

From equation [2], the slope of the regression of $Z_{1j}Z_{2j}$ on LD score (term $\frac{\sqrt{N_1N_2}\rho_g}{M}$) allows us to estimate genetic covariance between the two studies. On the other hand, the intercept (term $\frac{\rho N_s}{\sqrt{N_1N_2}}$) is affected by sample overlap and shared population stratification.

¹ Equations are adopted from Bulik-Sullivan et al., 2015 (13).

Genetic correlation (r_g) can be generated by normalising the genetic covariance, dividing it by SNP-heritability of trait 1 and trait 2, as summarised below:

$$r_g := \frac{\rho_g}{\sqrt{h_{SNP_1}^2 h_{SNP_2}^2}} \quad [3]^1$$

where $h_{SNP_1}^2$ is the SNP-heritability for trait 1 and $h_{SNP_2}^2$ is the SNP-heritability for trait 2.

The concept behind LD score regression is that the effect size estimate from a GWAS for a given SNP incorporates the effects of all SNPs that are in LD with that SNP. Therefore, for a trait that is genetically influenced, variants that tag more causal variants will have higher LD scores and therefore a higher test-statistics, in comparison to variants with lower LD scores (heteroscedasticity). The genome-wide inflation of the test-statistics, in turn, allows us to distinguish polygenicity from biases due to cryptic relatedness or population stratification, since such affect all SNPs equally, regardless of their LD score.

There are three major advantages of LD regression score. First, it only requires summary-level, not individual-level, genotype data unlike polygenic score analysis. Second, it is a method of high computational efficiency, and third, it is robust to sample overlap, as all variants are expected to be equally inflated regardless of the amount of genetic variation each SNP tags (B. Bulik-Sullivan et al., 2015). The latter is of particular importance, as controls are often shared between cohorts in GWA meta-analysis for different traits, and disentangling overlapping cohorts is challenging.

5.9 Limitation of the polygenic score and LD score regression approaches

Both polygenic score and LD score regression analysis are statistical tools which are developed to maximise the information available from

GWAS datasets to inform our understanding of the genetic architecture of complex disorders. There are, however, limitations to both approaches. For example, polygenic score approach includes contribution from SNPs with small effects sizes which do not reach genome-wide significance but given the missing heritability of T2DM and depression from GWAS, a non-significant finding could be a reflection of no association between the polygenic scores and trait being examined or a lack of power (type 2 error). We will use the statistical software package Additive Variance Explained and Number of Genetic Effects Method of Estimation (AVENGEME) (Dudbridge, 2013) to estimate the power of the UK Biobank sample (chapter 7).

It should also be noted that a SNP which is GWA significant for a trait might not be the causal variant. It can be a proxy for the causal variant, as the causal variant might not be genotyped or imputed because of LD. It might not, therefore, directly impact disease's susceptibility (indirect association). The threshold chosen for pruning the polygenic score approach is relatively stringent (pairwise r^2 threshold of 0.1 across 250kb). The process is commonly known as clumping, and its aim is to select SNPs that are most strongly associated with a trait in the region when constructing the polygenic scores but the thresholds set for clumping are arbitrary values. Thus, it is possible to retain multiple SNPs which are associated with the same causal variant while discarding correlated SNPs driven by independent causal variants (Wray et al., 2014). The polygenic score analysis is not currently designed to address this problem and studies have reported that SNPs which appear non-significant when examined independently could become highly significant when they are part of a pathway-based analysis (Peng et al., 2010).

Overfitting is another common criticism levelled towards the polygenic score approach. It refers to the scenario when a model is more complex than necessary and is a serious threat to the validity of a model. A model needs a sufficient number of parameters (complexity) to

encapsulate the underlying population relationship, but if the complexity grows beyond necessity, the model might start to capture noise rather than the true relationship. We will address the overfitting problem by restricting the number of P_T we examine in each dataset.

LD score regression, on the other hand, is not well suited to analysis of trans-ethnic or admixed populations. To generate LD scores, a reference dataset is required to robustly estimate the LD structure and there is currently limited data available for admixture scenarios. It also does not take into account the long-range admixture LD. Tools are currently being developed to estimate heritability for trans-ethnic or admixed populations (Brown, Ye, Price, & Zaitlen, 2016). This is an important development since GWAS have mostly focused on populations of Caucasian descent. In addition, trans-ethnic and fine-mapping have proved to be valuable avenues for identifying causal variants. Efforts have recently been made to extend methodology across populations with different ancestries (Brown et al., 2016). It will be interesting to examine the genetic overlap between T2DM and depression in non-Caucasian populations in the future.

GWAS uses a binary model for disease; cases and controls, but both T2DM and depression have shown extensive clinical and phenotypic heterogeneity. These variations could be due to environmental factors, population stratification or different biological processes underlying the two diseases. For this project, we are interested in whether T2DM and depression share any genetic aetiology but neither polygenic score nor LD score approaches can distinguish whether the sharing of SNPs across traits are driven by all individuals (pleiotropy) or a subset of individuals who are genetically more similar (heterogeneity). In addition, any phenotypic heterogeneity will dilute effect sizes, further reducing the power of a study. We will examine the genetic overlap between T2DM and depressive symptoms in order to explore phenotypic heterogeneity further (chapter 8). In addition, advanced statistical methods such as BUHMBOX

have recently been developed to facilitate interpretation of observed shared genetic architectures (Han et al., 2016).

5.10 Conclusion

GWA has provided us with a valuable way to study the genetic architecture underlying complex disorders. Various discoveries from autoimmune diseases to metabolic traits have been made through the experimental design of GWAS, with many of the detected loci being involved in biologically meaningful pathways for the diseases investigated (Visscher, Brown, McCarthy, & Yang, 2012). It has dramatically expanded our understanding of genetics of complex traits. Meta-analysis of multiple GWAS datasets has led to the identification of many genetic variants associated with common disorders. Given the small effect sizes of common genetic variants for complex traits, statistical methods such as polygenic score and LD score regression approaches have made important contributions in advancing our understanding of common disorders. Various methodological pitfalls remain and statistical genetics remain an exciting yet challenging field.

Chapter 6

Examining the T2DM and depression link using polygenic scores approach

“Pure and complete sorrow is as impossible as pure and complete joy.”

~ Leo Tolstoy, War and Peace

The dataset has been removed due to third party copyright material.

Chapter 7

Dissecting the genetic overlap between T2DM and depression in the UK Biobank

“Nothing thicker than a knife’s blade separates happiness from melancholy.”

~ Virginia Woolf, Orlando

Dissecting the genetic overlap between T2DM and depression in the UK Biobank

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7.1 Abstract

7.1.1 Background

Epidemiological studies have demonstrated an association between Type 2 diabetes (T2DM) and depression, and recent twin studies suggest a genetic overlap between the two disorders. The aim of this is to systematically examine the genetic overlap between T2DM and depression using two complementary approaches in the UK Biobank.

7.1.2 Methods

We tested whether depression-polygenic scores is associated with T2DM case status and T2DM-polygenic scores with depression case-status. We also estimated the genetic correlation between the two disorders using linkage disequilibrium (LD) score regression. Secondary analyses examined the effect of sex, body mass index (BMI) and severity of depression.

7.1.3 Results

No association between T2DM-polygenic scores and depression case status or depression-polygenic scores with T2DM case status was observed. The genetic correlation between the two disorders was also statistically non-significant.

7.1.4 Conclusion

There is no evidence of shared genetic susceptibility between T2DM and depression in the UK Biobank.

7.2 Background

Epidemiological studies have consistently reported an association between T2DM and depression, with up to 60% increased risk for incident T2DM in people with depression and 15% increased risk for developing depression in those with T2DM (Golden et al., 2008). Depression is associated with adverse effects on diabetes outcomes including suboptimal glycaemic control, complications and higher rates of mortality (De Groot et al., 2001; Katon et al., 2005; Lustman et al., 2000). Tentative evidence suggests these disorders share common pathophysiological mechanisms. For example, inflammatory markers, such as C-Reactive Protein, has been associated with depressive symptoms in individuals with newly diagnosed T2DM (Laake et al., 2014). A dysregulated and overactive hypothalamic–pituitary–adrenal axis has also been postulated as a possible biological explanation for the T2DM-depression association (Moulton, Pickup, & Ismail, 2015).

A genetic overlap between T2DM and depression has recently been reported in the Danish and Swedish twin registries. The T2DM-depression association is primarily attributed to genetic effects in both sexes in the Danish sample but this effect is only observed in females in the Swedish sample. In addition, qualitative sex differences in the genetic overlap between T2DM and depression was observed, suggesting that different genetic factors might be involved across the sexes in explaining the T2DM-depression association.

Both T2DM and depression are considered to be highly polygenic, whereby the overall genetic contribution to the traits results from the combined effects of many genetic variants exerting small individual effects (Ripke et al., 2013). Polygenic score analysis involves building genetic risk scores from the genetic components of one trait and examining its association with a second trait. Two recent studies using the polygenic score approach have concluded that there is little evidence of a genetic

overlap between T2DM and depression (Clarke et al., 2016; Samaan et al., 2015). There are, however, several caveats with both studies. The EpiDREAM study is a multinational, longitudinal study of 24872 individuals who were at risk for developing T2DM (9). It examines the unidirectional association between depression and 20 T2DM-single nucleotide polymorphisms (SNPs). Given that over 120 SNPs predisposing to T2DM and diabetes-related traits have been discovered, 20 SNPs are likely to capture only a small amount of the phenotypic variance for T2DM (Prasad & Groop, 2015). The Generation Scotland study is a family and population-based study with participants recruited from General Practitioners throughout Scotland. Genome-wide genotype data were available for 19858 individuals but only 915 individuals have a diagnosis of T2DM (Clarke et al., 2016). In addition, the effect of sex was not examined, despite a meta-analysis reporting a higher prevalence of depression in females with T2DM (23.8%) than males (12.8%; (Ali et al., 2006). In this study, we aim to investigate the genetic aetiology of the T2DM-depression association in the UK Biobank (Sudlow et al., 2015), using the polygenic scores and LD score regression approaches, in order to clarify whether a genetic predisposition to T2DM might be related to depression and vice versa.

7.3 Methods

7.3.1 Sample

The UK Biobank is a large, prospectively-sampled population cohort of 502656 adults aged 40-69, designed to examine the genetic and environmental determinants of diseases in adulthood (Sudlow et al., 2015). Full details on study design, survey methods and data collection has been reported elsewhere (UK Biobank, 2010). In brief, participants were selected through national patient registers based on distance from their nearest UK Biobank assessment centre. Demographic and phenotypic data was collected through extensive healthcare questionnaires,

interviews with nursing staff and electronic health records between 2006 and 2010. Biological samples were also collected for genotyping.

7.3.2 Primary variables derived from UK Biobank

T2DM case status was defined by self-reported medical diagnosis as previously described with slight modification (Peters, Huxley, & Woodward, 2016). In brief, an algorithm used the following information to define T2DM and to differentiate from type 1 diabetes: i) self-reported medical diagnosis of diabetes, ii) age at first diagnosis (≥ 30 years old for South Asian and African Caribbean and ≥ 36 years old for European) and iii) the use of oral anti-diabetic medications.

The presence of depression was based on either: i) a primary diagnosis of depression from the inpatient hospital episodes data (ICD 10 subchapters F32 and F33) or ii) a self-reported diagnosis as previously described (Smith et al., 2013). In brief, the latter case definition required a previous visit to a General Practitioners/psychiatrist for stress, anxiety or depression, and at least one period of depression/anhedonia lasting at least two weeks. Depression controls were defined as the absence of any hospitalisation/self-report diagnosis of depression, anxiety, bipolar disorder, psychosis, multiple personality disorder, autism or intellectual disability, and no previous usage of antidepressant, anxiolytic, mood stabilising or anti-psychotic medication.

7.3.3 Secondary variables derived from UK Biobank

Secondary variables included age, sex, ethnicity, BMI and previous history of severe depression. Demographic data were collected from baseline self-report questionnaires and BMI was derived from baseline physical measurements obtained by trained staff. Height was measured using a Seca 202 height measure and weight using a Tanita BC-418 MA body composition analyser. Severe depression is defined as having more than one episode of depression and receiving care from a psychiatrist during their lifetime as self-reported by participants.

7.3.4 Genotyping, imputation and quality control

The interim release of the genotyping data for UK Biobank consists of 152734 individuals. Genome-wide genotyping of the UK Biobank was performed by Affymetrix using two customised microarrays, the UK BiLEVE and UKB Axiom arrays. Both have similar content and assay over 800,000 genetic variants. Genotype data was imputed to a combined reference panel of the UK10K and 1000 Genomes Project Phase 3, using IMPUTE3 software. Full details on genotyping, quality control processes and imputation for the UK Biobank have been reported elsewhere (UK Biobank, 2015a, 2015b).

Additional quality control specific to this study was performed using standard genetic programs PLINK2 and QCTOOL (Chang et al., 2015; Tool). Stringent quality filters were applied, with SNPs being retained if they were common (minor allele frequency ≥ 0.01), imputed with high certainty (INFO Score ≥ 0.9) and did not deviate substantially from Hardy-Weinberg equilibrium (HWE $p \geq 1 \times 10^{-6}$). Participants were retained if they self-identified as of European ancestry, had more than 98% of genotyped variants available, and exhibited a genome-wide heterozygosity within three standard deviations (SDs) of the cohort mean.

7.3.5 Base datasets

Large genome-wide association studies (GWAS) are often conducted through meta-analysis of various individuals cohort studies and thus, only summary statistics, not individual genotypes, are often available in the public domain. Summary statistics from a GWAS consists of genotype information such as effect size, standard error and allele frequency for each SNP and can be used to create polygenic scores. Summary statistics from stage 1 of Diabetes Genetic Replication And Meta-analysis Consortium (DIAGRAM) version 3 was used to derive the T2DM-polygenic scores (Morris et al., 2012). It comprises of 12 cohorts, with 12171 diabetes cases and 56862 controls (Morris et al., 2012). The cohorts consist of individuals of European origin, drawn from Europe, USA and Australia. Cases were

individuals with a clinical diagnosis of T2DM according to the American Diabetes Association or World Health Organization criteria.

The latest summary statistics from the Psychiatric Genomics Consortium Major Depressive Disorder Phase 2 (PGC-MDD-29) study was used to derive the depression-polygenic scores. The PGC-MDD-29 comprises of 29 cohorts, with 16823 depression cases and 25632 controls (Ripke et al., 2013). The cohorts consist of individuals of European origin, drawn from Europe, USA and Australia. Cases were individuals with a lifetime diagnosis of major depressive disorder, ascertained through structured clinical interview, clinician-administered checklists or review of medical records.

7.3.6 Polygenic scores

From the SNP information in genome-wide summary statistics (p-value for association, odds ratio), polygenic risk scores were calculated using the software package PRSice v1.23 (Euesden et al., 2015). In brief, polygenic score is the sum of trait-associated alleles across many genetic loci, weighted by effect sizes estimated from a base GWAS (S. M. Purcell et al., 2009). T2DM and depression-polygenic scores were generated from DIAGRAM and PGC-MDD-29 (base datasets) respectively. SNPs that were in the relevant datasets and the UK Biobank dataset were retained and clumped to remove SNPs in strong LD, using the default options in PRSice. For each individual, T2DM and depression-polygenic scores were generated using SNPs with association p-values at seven threshold ($P_T < 0.001, 0.05, 0.1, 0.2, 0.3, 0.4$ and 0.5).

7.3.7 Statistical analysis

7.3.7.1 Logistic regression

Logistic regression was used to assess the association between i) T2DM-polygenic scores and depression case/control status and ii) depression-polygenic scores and T2DM case/control status. Covariates were included to adjust for genotyping batch, assessment centre and ten

ancestry-informative Principal Components (PC1-10), to address any potential confounding that arises from technical artefacts and population stratification.

Secondary analyses included examining the effect of i) sex, ii) BMI and iii) severity of depression. This is achieved by stratifying the sample by sex and by including an interaction term of sex and of BMI with each polygenic risk score. For statistically significance findings, models were also adjusted for interactions between covariate of interest (namely sex or BMI) and i) PC1-10, ii) genotyping batch, and iii) assessment centre, in order to further control for confounders (Keller, 2014). In addition, the association between T2DM-polygenic scores and severe depression case/control status was also examined.

From each model, we extracted the proportion of variance for T2DM/depression status explained by the polygenic risk score (*Nagelkerke's pseudo-R²*) and the *p*-value for the fit of the model. Given the potential of multiple testing for 7 *P_T* and 12 analyses, significance for each test was set at 0.0005 after Bonferroni correction (0.05/84). This is a conservative significance threshold, given that SNPs at different *P_T* are likely to be correlated. Post-hoc power calculations were performed in Additive Variance Explained and Number of Genetic Effects Method of Estimation (AVENGEME) (Palla & Dudbridge, 2015). It is a statistical package designed for power calculations of genetic models from polygenic scores. All statistical analyses were implemented in R (<http://www.r-project.org>).

7.3.7.2 LD score regression

LD score regression is a statistical method for estimating heritability and genetic correlation from summary GWAS statistics (B. K. Bulik-Sullivan et al., 2015). We therefore first conducted a GWAS for T2DM and depression in UK Biobank using the software package SNPTEST and then generated T2DM and depression summary statistics respectively. We implemented frequentist tests on probabilistic dosage

estimates from the imputed genotypes (Marchini & Howie, 2010). T2DM and depression were then separately regressed on the covariates from the logistic models (sex, age, assessment centre, birth cluster, deprivation index, batch and top 10 PCs) and the resulting residuals were used as the phenotype for GWAS.

In LD score regression, SNP-heritability for a trait is estimated by regressing the association statistic of a SNP on the average LD between the SNP of interest and proximal SNPs (the LD score). Genetic correlation between two traits can be estimated by regressing the product of the normalised association statistics for each SNP on the LD score. For this analysis, LD score is estimated from the European subset of the 1000 Genomes. LD score regression accounts for both LD-independent and LD-dependent errors and therefore avoids any bias arising from sample overlap (B. Bulik-Sullivan et al., 2015). This is of particular important, as we are estimating heritability and genetic correlation from the same dataset in this study.

7.4 Results

7.4.1 Demographics and analytical sample

The UK Biobank sample with genotyping data consisted of 152551 participants (80702 males; 71547 females), with a mean age of 56.6 years (SD: 8.0) and mean BMI of 27.6 kg/m² (4.9; Table 7-1). Restricting to samples of European ancestry, there was i) 5949 cases and 137763 controls for T2DM and ii) 10005 cases and 19314 controls for depression.

Table 7-1 Demographic characteristics of individuals with genotyping data from UK Biobank.

^ap-values were calculated using χ^2 for quantitative variables and t-test for discrete variables.

	Full sample	T2DM			Depression		
		Case	Control	<i>p</i> ^a	Case	Control	<i>p</i> ^a
N	152551	5949	137763		10005	19314	
Male	80702	3884	63543	<0.0001	3710	10878	<0.0001
N (%)	(52.9)	(65.2)	(46.1)		(37.1)	(56.3)	
Mean age, years (SD)	56.6 (8.0)	60.6 (6.5)	56.6 (8.0)	<0.0001	56.0 (7.8)	57.6 (8.0)	<0.0001
Mean BMI, kg/m² (SD)^b	27.6 (4.9)	32.1 (5.8)	27.3 (4.7)	<0.0001	27.8 (5.2)	27.3 (4.5)	<0.0001

7.4.2 Polygenic risk scoring

Both T2DM and depression-polygenic scores were strongly predictive of their corresponding disorders. The maximum variance explained was 0.99% for T2DM ($p < 10^{-92}$ at $P_T < 0.2$) and 0.25% for depression ($p < 10^{-13}$ at $P_T < 0.5$). T2DM-polygenic scores were not predictive of depression case status and depression-polygenic scores were not predictive of T2DM case status (Table 7-2).

Table 7-2 Association of i) depression-polygenic scores with T2DM in UK Biobank and ii) T2DM polygenic scores with depression at various P_T .

i) Association with T2DM				ii) Association with depression		
P_T	Depression-polygenic scores			T2DM-polygenic scores		
	OR (95% CI)	R^2 explained (%)	p	OR (95% CI)	R^2 explained (%)	p
P_T						
< 0.001	1.01 (0.99-1.04)	0.0252	0.303	1.00 (0.98-1.02)	0.0007	0.695
< 0.05	1.02 (0.99-1.05)	0.0051	0.142	1.00 (0.97-1.02)	0.0003	0.793
< 0.1	1.01 (0.99-1.04)	0.0026	0.296	1.00 (0.97-1.03)	2.3×10^{-5}	0.944
< 0.2	1.01 (0.98-1.04)	0.0013	0.458	1.00 (0.98-1.03)	0.0009	0.658
< 0.3	1.01 (0.98-1.04)	0.0012	0.470	1.00 (0.98-1.03)	0.0009	0.670
< 0.4	1.01 (0.98-1.04)	0.0016	0.417	1.00 (0.97-1.03)	3.8×10^{-6}	0.978
< 0.5	1.01 (0.98-1.04)	0.0012	0.472	1.00 (0.97-1.03)	4.1×10^{-5}	0.925

7.4.3 Secondary analysis

All findings were statistically non-significant after Bonferroni correction. Nominally significant results were observed in secondary analysis. When stratified by sex, an association between depression-polygenic scores and depression case status was observed in males only (R^2 explained=0.022%; $p=0.020$ at $P_T<0.1$; Table 7-3). The interaction between sex and depression-polygenic scores has an effect on T2DM case-status ($p=0.037$ at $P_T<0.1$; Table 7-4) whereas the interaction between BMI and T2DM-polygenic scores has an effect on depression case-status ($p=0.0009$ at $P_T<0.001$; Table 7-4). When sex and BMI were further controlled for cofounders respectively, such as sex \times genotyping batch, sex \times assessment centre and sex \times PC 1-10, the results remain marginally significant for i) sex and depression-polygenic scores on T2DM case-status ($p=0.027$ at $P_T<0.1$; results not shown) and ii) BMI and T2DM-polygenic scores on depression case-status ($p=0.009$ at $P_T<0.001$; results not shown).

The interaction between sex and T2DM-polygenic scores has no effect on depression case-status, nor the interaction between BMI and depression-polygenic scores on T2DM case-status (Table 7-4). T2DM-polygenic scores were not predictive of severe depression case-status (Table 7-5).

Table 7-3 Association of i) depression-polygenic scores with T2DM and ii) T2DM-polygenic scores with depression in UK Biobank, stratified by sex, at various P_T .

p-value significant at 0.05

i) Association with T2DM								
P_T	Depression-polygenic scores							
	Males 3884 cases; 63543 controls				Females 2065 cases; 74220 controls			
	OR (95% CI)	R^2 explained (%)	p		OR (95% CI)	R^2 explained (%)	p	
	< 0.001	1.01 (0.98-1.04)	0.0015		0.550	1.02 (0.97-1.06)	0.0034	0.450
	< 0.05	1.04 (1.01-1.07)	0.0212		0.024*	0.99 (0.94-1.04)	0.0011	0.668
	< 0.1	1.04 (1.01-1.07)	0.0224		0.020*	0.98 (0.93-1.02)	0.0074	0.265
	< 0.2	1.03 (0.99-1.06)	0.0104		0.113	0.98 (0.94-1.02)	0.0030	0.474
	< 0.3	1.02 (0.98-1.05)	0.0459		0.293	1.00 (0.96-1.05)	1.44 x 10 ⁻⁷	0.996
< 0.4	1.01 (0.98-1.05)	0.0030	0.396	1.01 (0.96-1.05)	0.0009	0.696		
< 0.5	1.01 (0.98-1.05)	0.0022	0.472	1.01 (0.97-1.06)	0.0011	0.668		

ii) Association with depression

	T2DM-polygenic scores						
	Males 3710 cases; 10878 controls				Females 6295 cases; 8436 controls		
	OR (95% CI)	R ² explained (%)	p		OR (95% CI)	R ² explained (%)	p
P _T							
< 0.001	1.00 (0.96-1.04)	5.7 x 10 ⁻⁶	0.981		1.01 (0.98-1.04)	0.0030	0.562
< 0.05	1.00 (0.96-1.04)	4.1 x 10 ⁻⁴	0.840		1.00 (0.96-1.04)	1.2 x 10 ⁻⁷	0.997
< 0.1	0.99 (0.95-1.03)	0.0011	0.743		1.01 (0.97-1.05)	0.0027	0.582
< 0.2	1.01 (0.97-1.05)	0.0031	0.581		1.00 (0.97-1.04)	0.0004	0.838
< 0.3	1.01 (0.97-1.05)	0.0023	0.634		1.00 (0.97-1.04)	0.0005	0.810
< 0.4	1.01 (0.97-1.05)	0.0021	0.648		0.99 (0.96-1.03)	0.0010	0.745
< 0.5	1.01 (0.97-1.05)	0.0029	0.592		0.99 (0.96-1.03)	0.0011	0.732

Table 7-4 Association of the interaction between i) sex and depression-polygenic scores with T2DM, ii) sex and T2DM-polygenic scores with depression, iii) BMI and depression-polygenic scores with T2DM, iv) BMI and T2DM polygenic scores with depression in UK Biobank, at various P_T .

p -value significant at <0.05 ; ** p -value significant at <0.001

i) Association with T2DM				ii) Association with depression			
P_T	Interaction between sex and depression-polygenic scores				Interaction between sex and T2DM-polygenic scores		
	β	SE	p		β	SE	p
P_T							
< 0.001	-0.0088	0.0278	0.752		-0.0052	0.0254	0.838
< 0.05	0.0424	0.0278	0.127		0.0070	0.0254	0.784
< 0.1	0.0578	0.0278	0.037*		-0.0018	0.0253	0.944
< 0.2	0.0367	0.0278	0.186		0.0190	0.0253	0.454
< 0.3	0.0119	0.0278	0.668		0.0175	0.0253	0.491
< 0.4	0.0003	0.0278	0.992		0.0260	0.0253	0.305
< 0.5	-0.0028	0.0278	0.921		0.0277	0.0253	0.275

iii) Association with T2DM				iv) Association with depression			
<i>P_T</i>	Interaction between BMI and depression-polygenic scores				Interaction between BMI and T2DM- polygenic scores		
	β	SE	<i>p</i>		β	SE	<i>p</i>
	< 0.001	-2.5 x 10 ⁻³	0.0022		-0.0086	0.0026	0.0009**
	< 0.05	0.0036	0.0022		-0.0058	0.0026	0.0267*
	< 0.1	0.0019	0.0022		-0.0064	0.0026	0.0148*
	< 0.2	0.0028	0.0022		-0.0050	0.0026	0.0579
	< 0.3	0.0021	0.0022		-0.0045	0.0026	0.0851
	< 0.4	0.0026	0.0022		-0.0037	0.0026	0.155
	< 0.5	0.0024	0.0022		-0.0038	0.0026	0.147

Table 7-5 Association of T2DM-polygenic scores with severe depression in UK Biobank, at various P_T .

Association with severe depression		
Cases of severe depression: 2607; controls: 19314		
	T2DM-polygenic scores	
	R ² explained (%)	p
P_T		
< 0.001	<0.0001	0.912
< 0.05	<0.0001	0.504
< 0.1	<0.0001	0.749
< 0.2	<0.0001	0.655
< 0.3	<0.0001	0.845
< 0.4	<0.0001	0.916
< 0.5	<0.0001	0.871

7.4.4 LD score regression

We also estimated the genetic correlation between T2DM and depression using LD score regression as it is protected against bias due to sample overlap and population stratification. Summary statistics for T2DM and depression was first created from UK Biobank. SNP-heritability on the liability scale was 0.20 (95% confidence interval (CI): 0.14-0.24) for T2DM and 0.10 (95% CI: 0.06-0.15) for depression, assuming population prevalence of 8% and 12% for T2DM and depression respectively.

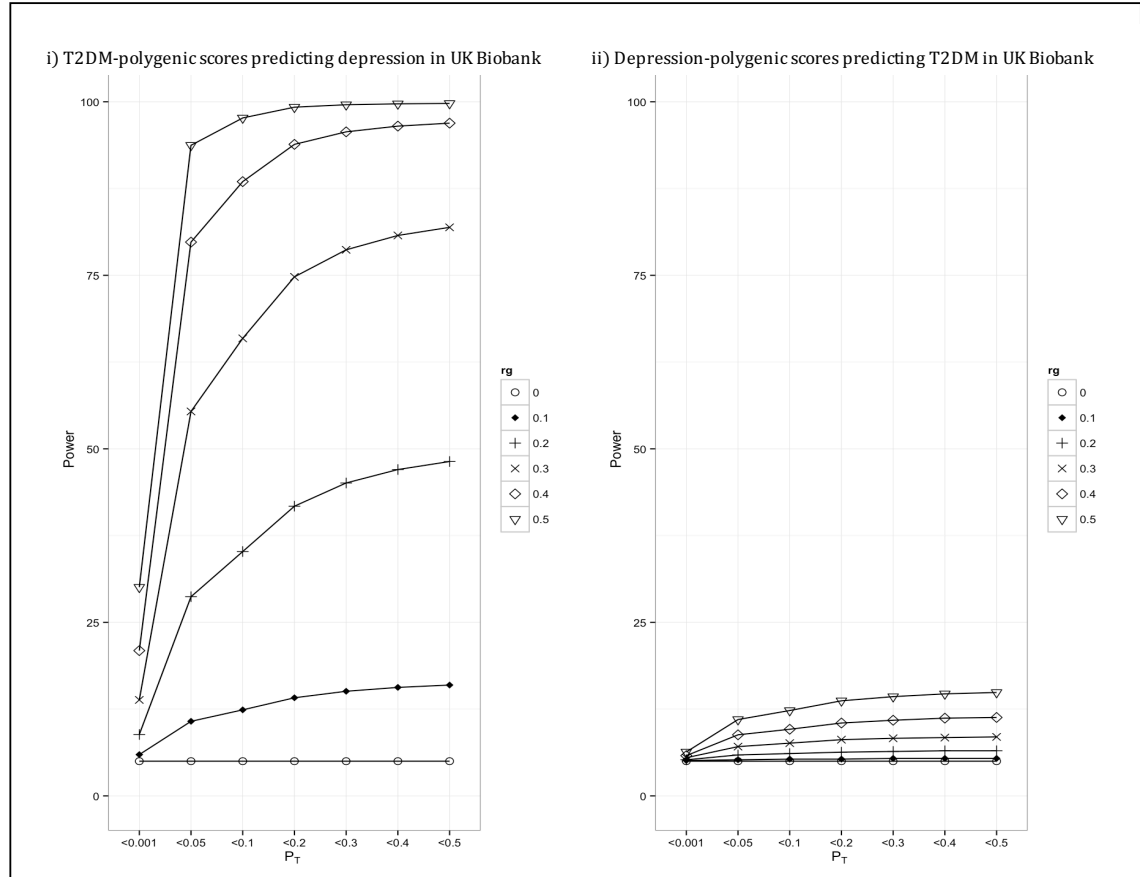
The phenotypic correlation between T2DM and depression was non-significant ($r=0.03$; 95% CI: 0-0.07; $p=0.07$). This needs to be interpreted in the context of a low prevalence of T2DM in the sample (5949 T2DM cases; 137763 controls; 4.1% of T2DM cases) and the depression controls were screened for psychiatric disorders (10005 depression cases; 19314 controls; 34.1% of depression cases). The genetic correlation using LD score regression was statistically non-significant ($r_g=-0.08$; 95% CI: -0.30-0.14; $p=0.474$).

7.4.5 Power calculation

In post-hoc power calculation using AVENGEME, the power of polygenic risk scores was 41.7% between T2DM-polygenic scores and depression (at $P_T < 0.2$, $\alpha=0.05$ and genetic correlation was assumed at 0.2 using estimates derived from twin studies (Kan et al., 2016) and 6.3% between depression-polygenic scores and T2DM (at $P_T < 0.2$ and genetic correlation estimated at 0.2), assuming population prevalence of 8% and 12% for T2DM and depression respectively and 5% of SNPs markers having an effect on T2DM/depression (Figure 7-1).

Figure 7-1 Power as a function of p -value thresholds for selecting SNP markers (P_T) into i) T2DM-polygenic scores in predicting depression and ii) depression-polygenic scores in predicting T2DM, at various genetic correlation (r_g) and $\alpha=0.05$.

Assumptions: population prevalence of 8% for T2DM and 12% for depression; 5% of SNPs markers having an effect on T2DM/depression.



7.5 Discussion

To our knowledge, this is the largest study to date that explores the genetic overlap between T2DM and depression, using the polygenic score approach at a population level. T2DM-polygenic scores were not associated with depression, nor depression-polygenic scores with T2DM. The genetic correlation between T2DM and depression was negative and statistically not different from zero. Our study therefore did not support the hypothesis of a genetic overlap between T2DM and depression in the

UK population. Using the LD score regression approach, we reported a non-significant, negative genetic correlation between T2DM and depression. This is similar to the finding from a recent analysis of the summary statistics from DIAGRAM and an earlier version of PGC-MDD whereby a non-significant, positive genetic correlation for T2DM and depression was reported (r_g (standard error (SE)): 0.051 (0.117), $p=0.666$) (B. Bulik-Sullivan et al., 2015).

The negative finding differs from previous twin studies whereby a genetic overlap between T2DM and depression was observed. A possible explanation for the difference is that the classic twin studies capture additive and non-additive effects of genetic factors across the allelic spectrum, but a GWAS using commercial microarrays predominantly captures common variants. Methods relying on GWAS data therefore do not directly detect the effect of uncommon variants, copy number variants, parental/maternal transmission and epistasis. This is in part reflected in the difference between the variance explained by GWAS SNPs (SNP-heritability) and heritability estimates from twin studies for T2DM and depression. For example, the heritability for depression is estimated to be 37% in twin studies (Flint & Kendler, 2014) whereas it is 14% from the latest PGC-MDD-29 (Ripke et al., 2013). Our findings should therefore be interpreted in the context of this missing heritability, although a recent study has suggested that low frequency and rare variants are unlikely to be an important source of contribution to the heritability of T2DM in comparison to common variants (Fuchsberger et al., 2016).

With recent advances in whole-genome sequencing technologies, the proportion of genetic variation captured by genotyped data should eventually be closer to ones from twin studies (Yang et al., 2015). The genetic correlations between T2DM and depression observed from twin studies might reflect the upper bounds of the variance in liability attributable to genetic factors, since heritability estimates derived from twin studies are consistently higher than those from GWAS (Manolio et

al., 2009). In addition, the equal environment assumption whereby MZ and DZ twins are correlated for shared environmental influences to the same extent has been challenged. Prenatal development process might lead MZ twins being epigenetically more similar than DZ twins, potentially overestimating the heritability component (Golan et al., 2014). Further research is needed to clarify this hypothesis and its effect.

It is also possible that the observed co-heritability between T2DM and depression in twin studies is attributable to aetiological diversity, with a subset of individuals with one disease being genetically similar to individuals with another disease (Wray, Lee, & Kendler, 2012). For example, a recent study with 3230 participants from the Netherlands Study of Depression and Anxiety and Netherlands Twin Registry has reported a genetic overlap between BMI and atypical depression, but not typical depression, defined as changes in appetite/weight (Milaneschi et al., 2015). Of note, the twin studies reporting significant genetic overlap between T2DM and depression utilise their respective national hospital discharge registries in their case definition for T2DM and depression (Kan et al., 2016). These do not include visits to general practitioners and its authors therefore noted that it might capture more severe cases. In our study, T2DM-polygenic scores were not predictive of depression case status when case status is restricted to individuals with severe depression. The sample size is, however, reduced substantially (cases: 2607; controls: 19314).

Our secondary analysis suggests that sex might play a role in the effect of depression-polygenic scores on T2DM case status, and BMI with T2DM-polygenic scores on depression case-status. The results were not statistically significant after multiple testing corrections. These findings need to be interpreted with caution since it is possible that a proportion of individuals in the base dataset (DIAGRAM) is also in the target dataset (UK Biobank). We were able to ascertain this possibility for depression but not for T2DM, as we only have access to the summary statistics of

DIAGRAM. The likely effect of sample overlap between base and target datasets is inflation in variance explained and might therefore explain the spurious association of the interaction between BMI and T2DM-polygenic scores on depression case-status in the secondary analysis. Replication of our finding in an independent dataset will address sample overlap between base and target datasets. Within the limits of our methodology, it is currently not possible to conclude the significance of the effect observed in our data.

7.5.1 Limitation

Both T2DM and depression-polygenic scores show statistically significant predictive ability for their respective disorders in UK Biobank, but they only explain a small amount of the phenotypic variance observed (T2DM: 0.99%; depression: 0.25%). This is reflected in limited power of association testing between T2DM and depression-polygenic scores (41.7%) and depression and T2DM-polygenic scores (6.3%). Our study is well powered for examining the genetic overlap between T2DM and depression if the genetic correlation between T2DM and depression is ~ 0.4 (power=93.9% at $P_T < 0.2$ and $\alpha = 0.05$).

Given the sex-specific genetic correlations reported from twin studies are ~ 0.2 , our study is under powered despite utilising the largest population cohort for T2DM and depression to date. Future meta-analysis on the genetic overlap between T2DM and depression in large population cohorts and utilising larger base datasets to generate polygenic scores could potentially address the pitfall of type-II errors.

A further limitation is the reliance on self-reported data for identifying individuals with T2DM and depression. Some misclassification of diabetes status could have occurred, but this is likely to be moderate, given T2DM covers 90% of adults with diabetes (Alberti & Zimmet, 1998). The UK Biobank has recently collected additional data on depressive symptoms, using the Composite International Diagnostic Interview Short Form (Kessler, Andrews, Mroczek, Ustun, & Wittchen, 1998). It should be

available in early spring 2017, allowing us to examine the genetic overlap between T2DM and specific depressive symptom. More accurate phenotyping, such as the use of repeated measures and semi-structured interviews, could potentially improve the heritability estimates for T2DM and depression.

7.6 Conclusion

Both T2DM and depression are likely due to a combination of genetic and environmental factors. The current study is unparalleled in its sample size for polygenic score analysis. Our finding of no genetic overlap between T2DM and depression contrasts with the positive findings from twin studies (Kan et al., 2016). Further work is needed to understand the reasons underling the discrepancy in findings, allowing better understanding of pathogenesis underlying both disorders.

7.7 Acknowledgments

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Chapter 8

Polygenic dissection of T2DM and depressive symptoms

But as, in ethics, evil is a consequence of good, so, in fact, out of joy is sorrow born. Either the memory of past bliss is the anguish of today, or the agonies which are have their origin in the ecstasies which might have been.”

~ Edgar Allan Poe, Berenice

Polygenic dissection of T2DM and depressive symptoms

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Abstract: 219

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Table: 4

Figure: 7

Key words: T2DM, depressive symptoms, heterogeneity, genetic, polygenic risk scores

8.1 Abstract

8.1.1 Background

The association between type 2 diabetes (T2DM) and depression has been consistently reported in epidemiological studies. The T2DM-depression link may be symptom-specific. We therefore examined whether polygenic risk scores for T2DM predict specific depressive symptoms in the Psychiatric Genomics Consortium Major Depressive Disorder Phase 2 (PGC-MDD-29).

8.1.2 Method

T2DM-polygenic scores were constructed from the association summary statistics of Diabetes Genetic Replication And Meta-analysis Consortium (DIAGRAM; 12171 cases and 56862 controls), at seven association p-value thresholds ($P_T < 0.0001-0.5$). Stepwise regression was used to test for association between T2DM-polygenic scores at $P_T < 0.5$ and specific depressive symptoms in PGC-MDD-29 (2360 cases and 4182 controls), adjusting for principal components (PCs) and study. Secondary analyses involved stratification by depression case-control status and sex.

8.1.3 Results

Feelings of guilt/worthlessness is the only significant depressive symptom predictor for T2DM-polygenic scores at $P_T < 0.5$ ($\beta = -0.00004$; $p = 0.0037$). The association remains significant for T2DM-polygenic scores between $P_T < 0.1$ and $P_T < 0.4$. When stratified by depression case-control status and sex, feelings of guilt/worthlessness is the only significant depressive symptom predictor for T2DM-polygenic scores at $P_T < 0.5$ in depression cases ($p = 0.0003$).

8.1.4 Conclusion

This study provides the first evidence of a shared genetic aetiology between T2DM and feelings of guilt/worthlessness, particularly in people with depression. Our finding tentatively supports that the epidemiological

association between T2DM and depression might have a genetic underpinning.

8.2 Background

Both T2DM and depression are common global disorders, with depression being associated with both insulin resistance (Kan et al., 2013) and T2DM (Golden et al., 2008). Individuals with depression have worse glycaemic control, greater diabetes complications and higher rates of mortality (Black, Markides, & Ray, 2003; Lustman et al., 2000). Treatment of depression does not always lead to improvement in glycaemic control (Markowitz, Gonzalez, Wilkinson, & Safren, 2011). There is accumulating evidences that substantial heterogeneity exists in the psychopathology of depression (Harald & Gordon, 2012). Data-driven approaches have reported the existence of symptoms-based subtypes, namely atypical and typical depression (Alexandrino-Silva et al., 2013; Lamers et al., 2013; Li et al., 2014). The former is often characterised by the presence of increased appetite/weight and/or hypersomnia and is associated with adiposity measures, such as body mass index (BMI), waist circumference and incidence of obesity (Lasserre et al., 2014). Given visceral obesity has been postulated as a biological explanatory link for the T2DM-depression association (Champaneri et al., 2010; Golden et al., 2008), certain depressive subtypes might be associated with T2DM, suggesting the existence of a subset of individuals with common pathophysiology.

Inflammation has also been reported to be involved in the pathogenesis of the T2DM-depression link, with inflammatory markers, such as C-Reactive Proteins, being associated with depressive symptoms in individuals with newly diagnosed T2DM (Laake et al., 2014). Of interest, association between inflammatory biomarkers and depression has been suggested to be symptom-specific. For example, a recent study reported that elevated levels of CRP are associated with specific depressive

symptoms, namely change in appetite, sleep disturbances and fatigue in the general population (Jokela, Virtanen, Batty, & Kivimaki, 2016).

Twin studies have suggested qualitative and quantitative sex differences in the shared genetic aetiology between T2DM and depression (Kan et al., 2016) but no genetic overlap has been observed in GWAS datasets using polygenic scores and LD score regression approaches (B. Bulik-Sullivan et al., 2015; Clarke et al., 2015; Samaan et al., 2015). The underlying genetic aetiology between T2DM and depression is currently unclear. Previous research examining T2DM and depression has, however, assumed depression is a homogeneous condition. Given the large phenotypic and aetiological heterogeneity of depression and variation in response to treatment of depression, it might be useful to examine whether there is a genetic overlap between T2DM and specific depressive symptoms, rather than studying depression as a whole. The value of studying depression subtypes in understanding the genetic overlap with physical disorders was seen in a recent study with 3230 participants from the Netherlands Study of Depression and Anxiety and Netherlands Twin Registry (Milaneschi et al., 2015). It reported a genetic overlap between BMI-polygenic scores and atypical depression, defined as increased appetite/weight (Milaneschi et al., 2015). In this study, we aim to determine whether there is an association between T2DM-polygenic scores and specific depressive symptoms in PGC-MDD-29.

8.3 Methods

8.3.1 Sample

The PGC-MDD is an on-going international collaboration with the aim of understanding the genetics architecture of major depressive disorder. The latest dataset consists of 29 research studies, with 16823 depression cases and 25632 controls (Ripke et al., 2013). In brief, it comprises individuals of European descents from Europe, USA and Australia. Cases were individuals with a lifetime diagnosis of major

depressive disorder as defined in the Diagnostic and Statistical Manual of Mental Disorder-4th Edition (DSM-IV). Diagnostic information was ascertained through structured clinical interview, clinician-administered checklists or review of medical records. Controls were mainly selected from the population and screened for lifetime depression. Cohorts included in both stage 1 of DIAGRAM and in PGC-MDD-29 were excluded from analysis. In addition, we excluded any cohort whereby depressive symptoms were available for depression cases or controls only or have less than 50 individuals providing information on specific depressive symptoms. This led to a total of 4 cohorts, with 2360 cases and 4182 controls with sufficient information on genotype and depressive symptoms for analysis.

8.3.2 Depressive symptoms

Depressive symptoms were defined according to Diagnostic and DSM 4th Edition as followed: i) low mood, ii) anhedonia (a loss of interest or pleasure in daily activities), iii) changes in appetite/weight, iv) sleep disturbances, v) psychomotor disturbances, vi) fatigue, vii) feelings of guilt/worthlessness, viii) poor concentration and ix) suicidality. All symptoms were defined as either “absent” or “present”, including symptoms with bidirectional response, namely: i) changes in appetite/weight, ii) sleep disturbances and iii) psychomotor disturbances. They were defined as “absent” or “changes in either direction”.

8.3.3 Genotyping and imputation

The quality control procedures adopted in PGC-MDD-29 analysis are applied to all PGC studies, and have been described in details elsewhere (Ripke et al., 2013). In brief, the PGC designed the “RicoPili” pipeline (<https://github.com/Nealelab/ricopili/wiki>) to process all individual-level genotype data. It is used for quality control, imputation and analysis of genotype data, ensuring uniform procedures across all cohorts. Empirical relatedness was computed from genotype data and one of each duplicated/related pair, defined as $\pi > 0.2$, was excluded. The

quality control applied per cohort included: i) minor allele frequency ≥ 0.01 , ii) Hardy-Weinberg equilibrium $p \geq 0.0001$ and iii) imputation marker information content metric (INFO) score ≥ 0.6 . For imputation, the 1000 Genomes Project multi-ancestry reference panel was used (Abecasis et al., 2010). Ancestry informative PCs were calculated for each study in PGC-MDD-29, allowing us to control for the effect of population stratification in statistical analysis. Imputed data converted to hard-called genotypes was used to build the T2DM-polygenic scores.

8.3.4 Base dataset

T2DM-polygenic scores are created from the summary statistics from stage 1 of DIAGRAM version 3 (Morris et al., 2012). It comprises of 12 cohorts, with 12171 diabetes cases and 56862 controls (Morris et al., 2012). The cohorts consist of individuals of European descents from Europe, USA and Australia. Cases were individuals with a clinical diagnosis of T2DM according to the American Diabetes Association or World Health Organization criteria.

8.3.5 Polygenic scores

T2DM-polygenic risk scores were calculated using the software package PRSice v1.23 (Euesden et al., 2015). In brief, polygenic score is the sum of trait-associated alleles across many genetic loci, weighted by effect sizes estimated from a base GWAS (S. M. Purcell et al., 2009). T2DM-polygenic scores were generated from Stage 1 of DIAGRAM version 3 (base dataset). SNPs that were in both DIAGRAM and PGC-MDD-29 were retained and clumped to remove SNPs in strong LD, using the default options in PRSice. For each individual in PGC-MDD-29, T2DM-polygenic scores were generated using SNPs with 7 P_T (<0.001 , 0.01, 0.1, 0.2, 0.3, 0.4 and 0.5).

8.4 Statistical analysis

To assess the level of correlation between depressive symptoms, we first calculated tetrachoric correlations between the symptoms. We then used Principal component analysis (PCA) to dissect and capture the underlying structure between depressive symptoms. PCA is a statistical method that reduces a set of possibly correlated variables into linearly uncorrelated variables called PCs (Jolliffe, 2002). It therefore reduces dimensionality of a dataset while retaining most of its variation (Ringner, 2008). The number of PCs retained for further analysis was determined according to Kaiser criterion (Kaiser, 1960), whereby PCs with eigenvalues ≥ 1 were retained.

It should, however, be noted that PCA is not designed to identify directions for separating classes of samples. Biplot is often used to visualised the results from PCA. It extends the idea of a simple scatterplot of two variables to multiple variables, allowing maximum visualisation of information in a dataset. In a biplot, the rows and columns of the target matrix are visualized by their scalar product (Greenacre & Primicerio, 2013). Scatterplots and biplot of the first and second PC were plotted to identify potential sources of data variation, such as i) depression case-control status, ii) sex, iii) study and iv) specific depressive symptoms.

Stepwise linear regression was then used to assess the association between T2DM-polygenic scores and nine depressive symptoms as described under “depressive symptoms”. The outcome of interest is T2DM-polygenic scores at $P < 0.5$. We built a model by successively adding or removing explanatory variables, namely specific depressive symptoms, based on the Akaike information criterion, allowing us to fine-tune the model. The first six ancestry-informative PCs from the PGC-MDD 29 meta-analysis, PCs retained from the depressive symptoms PCA analysis and an indicator for each study were included as covariates, in order to address any potential confounding that arises from population

stratification and study artefacts (results not reported for these explanatory variables). A predictor is considered statistically significant for the stepwise linear regression if p -value is <0.01 .

The best fitting model was then tested with T2DM-polygenic scores at the remaining 6 P_T (< 0.001 , 0.01 , 0.1 , 0.2 , 0.3 and 0.4). For each model, we extracted the standardised regression coefficients (β), the proportion of variance in T2DM-polygenic scores explained (*Nagelkerke's pseudo- R^2*) and the p -value for each explanatory variable. Secondary analyses included examining the effect of depression case-status and sex by sample stratification. All statistical analyses were implemented in R (<http://www.r-project.org>).

8.5 Results

8.5.1 Descriptive analysis

The four cohorts from PGC-MDD-29 included in this study were: i) CoFaMS (Air, Weightman, & Baune, 2015), ii) PsyCoLaus (Preisig et al., 2009), iii) NESDA (Penninx et al., 2008) and iv) SHP0 (Volzke et al., 2011). In brief, CoFaMS is a case-control study and participants were recruited from the general community and clinical services in Australia, with depressive symptoms assessed by Mini Neuropsychiatric Diagnostic Interview 6.0 (Sheehan et al., 1998) and Hamilton Depression and Anxiety Scales (Williams, 1988). PsyCoLaus is a population-based study and participants were recruited from the population registry in Switzerland, with depressive symptoms assessed by Diagnostic Interview for Genetic Studies (Nurnberger et al., 1994) with additional questions added to elicit atypical depression features. NESDA is a naturalistic, longitudinal cohort study and participants were recruited from various health care settings, ranging from community to specialised mental health care in The Netherlands, with depressive symptoms assessed by Composite Interview Diagnostic Instrument (Wittchen, 1994) and Inventory of Depressive Symptoms (Rush et al., 1996). SHP0 is a population-based project and

participants were recruited from the residence registries in Germany, with depressive symptoms assessed by Mini-Mental Test (Folstein, Folstein, & McHugh, 1975).

In total, these consisted of 6542 participants (2838 males; 3704 females) of European ancestry, with a mean age at interview of 46.7 years (SD: 14.3; Table 8-1). There were 2360 cases and 4182 controls for depression. The number of individuals with response for each depressive symptom is summarised in table 2. Substantial tetrachoric correlations exist between the depressive symptoms (Figure 8-1).

Table 8-1 Demographic characteristics by study in PGC-MDD-29.

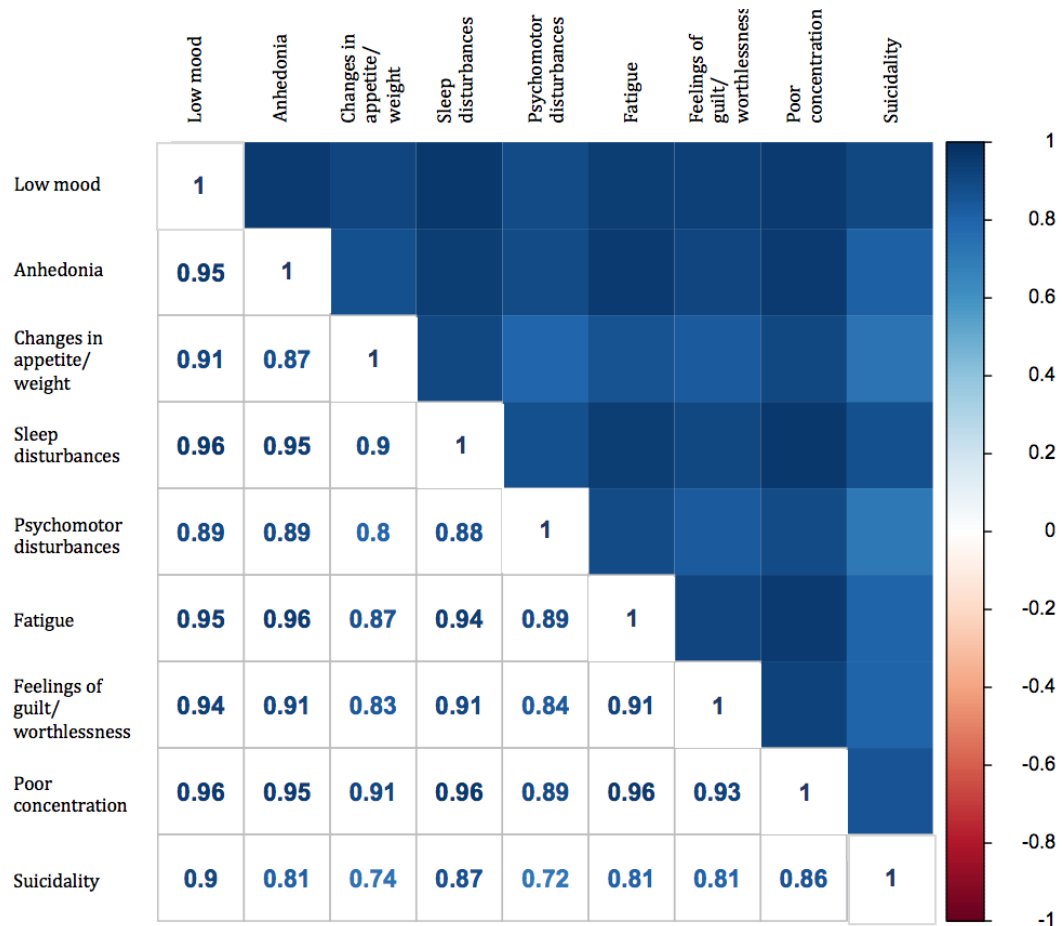
Study	Country	Total	Age at interview, mean (SD)	Number of males (%)	Number of depression cases (%)
CoFaMS	Australia	233	35.3 (17.0)	103 (44.2)	115 (49.4)
PsyCoLaus	Switzerland	1972	51.5 (8.8)	983 (49.8)	509 (25.8)
NESDA	The Netherlands	2912	40.0 (13.4)	1035 (35.5)	1375 (47.2)
SHP0	Germany	1505	55.4 (13.4)	757 (50.3)	373 (24.8)

Table 8-2 Depressive symptoms of individuals by study in PGC-MDD-29.

		Number of individuals reporting the presence of individual depressive symptom (%)								
Study	Total	Low mood	Anhedonia	Changes in appetite/weight	Sleep disturbances	Psychomotor disturbances	Fatigue	Feelings of guilt/worthlessness	Poor concentration	Suicidality
CoFaMS	227	102	102	92	100	93	102	99	98	82
		44.9%	44.9%	40.5%	44.1%	41.0%	44.9%	43.6%	43.2%	36.1%
PsyCoLaus	1972	837	688	391	595	512	657	531	513	290
		42.4%	34.9%	19.8%	30.2%	26.0%	33.3%	26.9%	26.0%	14.7%
NESDA	2912	1214	1192	939	1216	841	1228	1056	1304	688
		41.7%	40.9%	32.2%	41.8%	28.9%	42.2%	36.3%	44.8%	23.6%
SHPO	1505	366	252	264	344	184	202	209	281	261
		24.3%	16.7%	17.5%	22.9%	12.2%	13.4%	13.9%	18.7%	17.3%

Figure 8-1 Tetrachoric phenotypic correlation matrix across depressive symptoms in PGC-MDD-29.

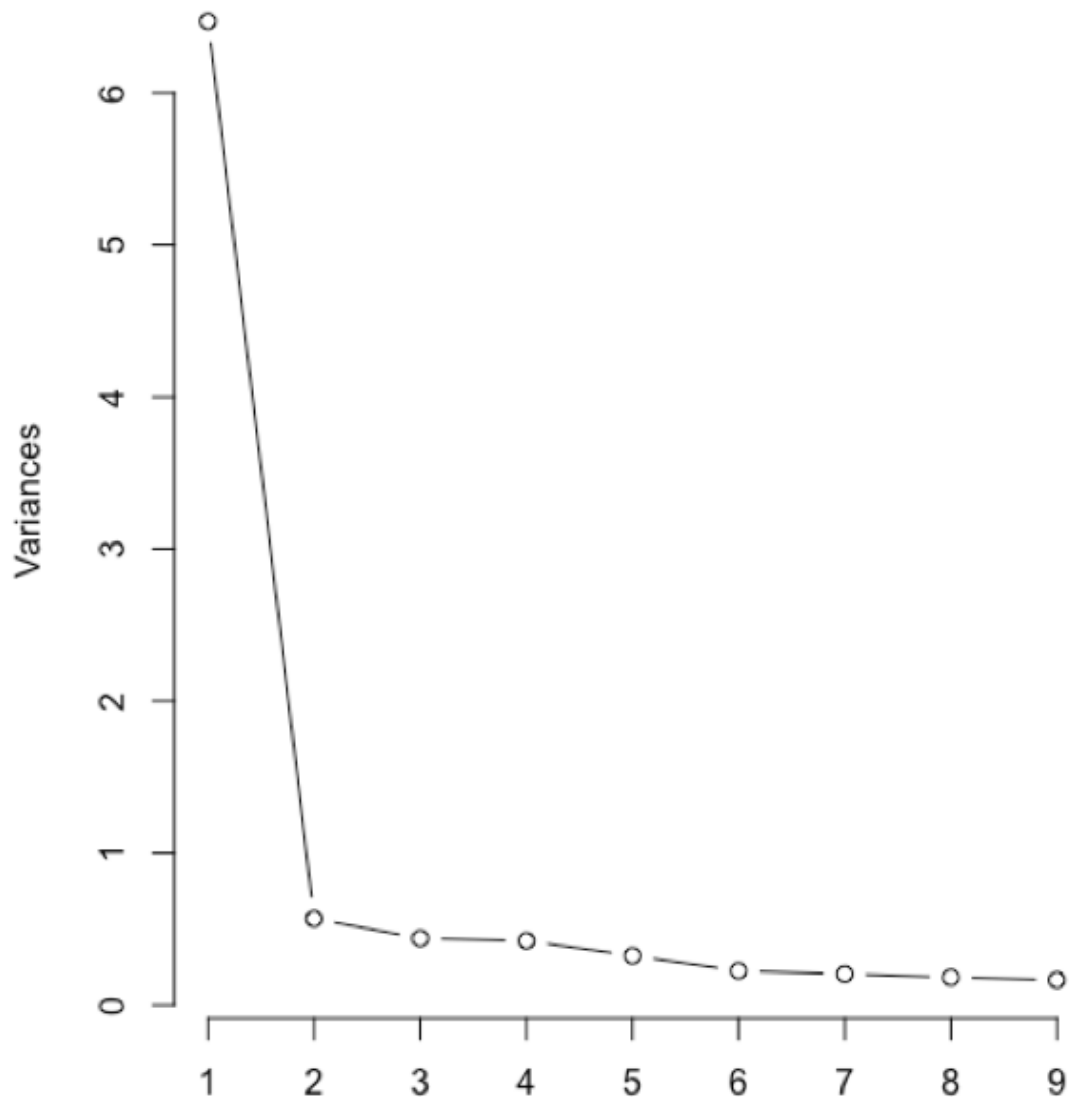
Colour of each box indicates the magnitude of the correlation, size of the boxes indicates its significance.



8.5.2 PCA analysis of depressive symptoms

The first PC explains 71.9% of the original variance in the nine depressive symptoms whereas the second PC explains 6.3% of the variance. The first PC is the only PC with eigenvalues ≥ 1 and is therefore retained for further analysis (Figure 8-2).

Figure 8-2 Proportion of variance fit by each PC in PGC-MDD-29.

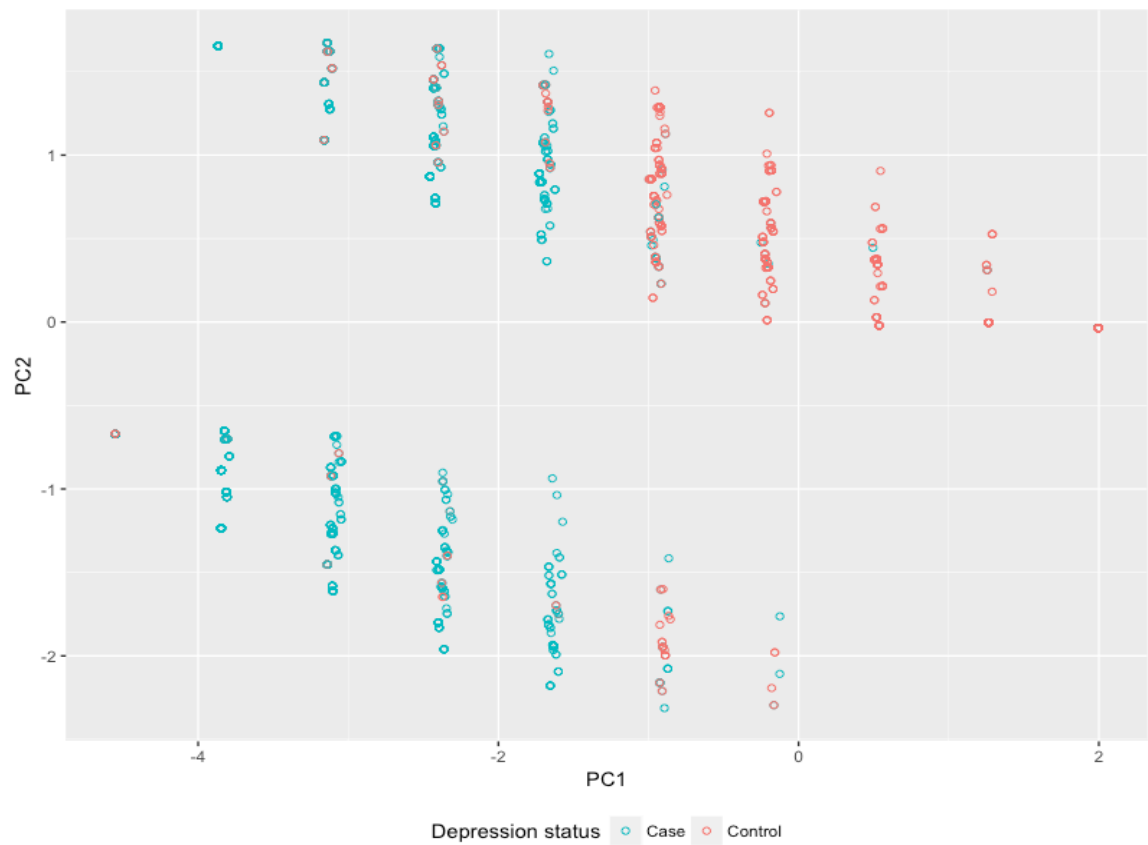


Visual inspection of the scatterplots suggests that a potential source of data variation for PC1 was depression case-control status while sex and study did not explain much of the data variation for PC2 (Figure 8-3). In the biplot of PC1 verses PC2, the points represent depressive symptoms and points which cluster together have similar profiles on the PCs (Figure 8-4). The biplot suggests that a potential source of data variation for PC2 is suicidality and the remaining 8 depressive symptoms. Linear regression demonstrates that 75.6% of the variation in PC1 was accounted by depression case-control status whereas 83.8% of the variation in PC2 was accounted by suicidality.

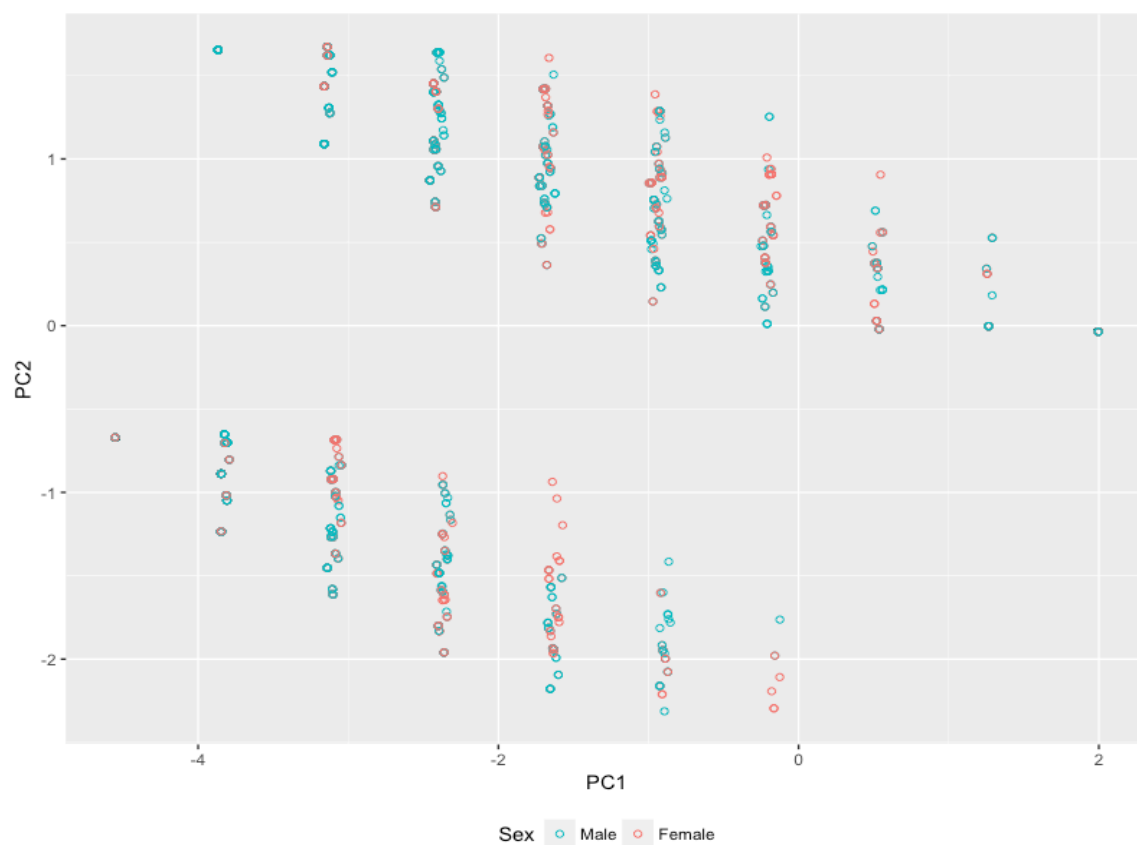
Figure 8-3 Scatterplots of PCA in PGC-MDD-29.

Datasets are colour coded to show the inherent clustering of datasets by i) depression status, ii) sex and iii) study.

i)



ii)



iii)

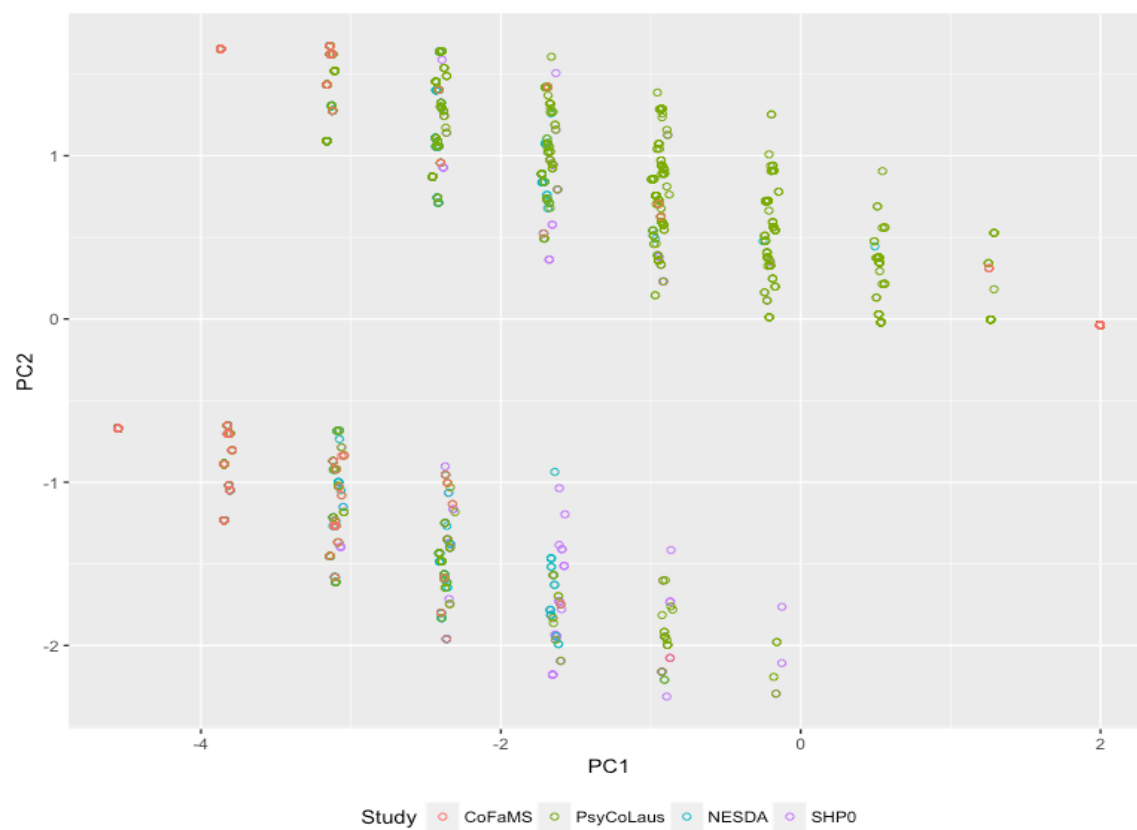
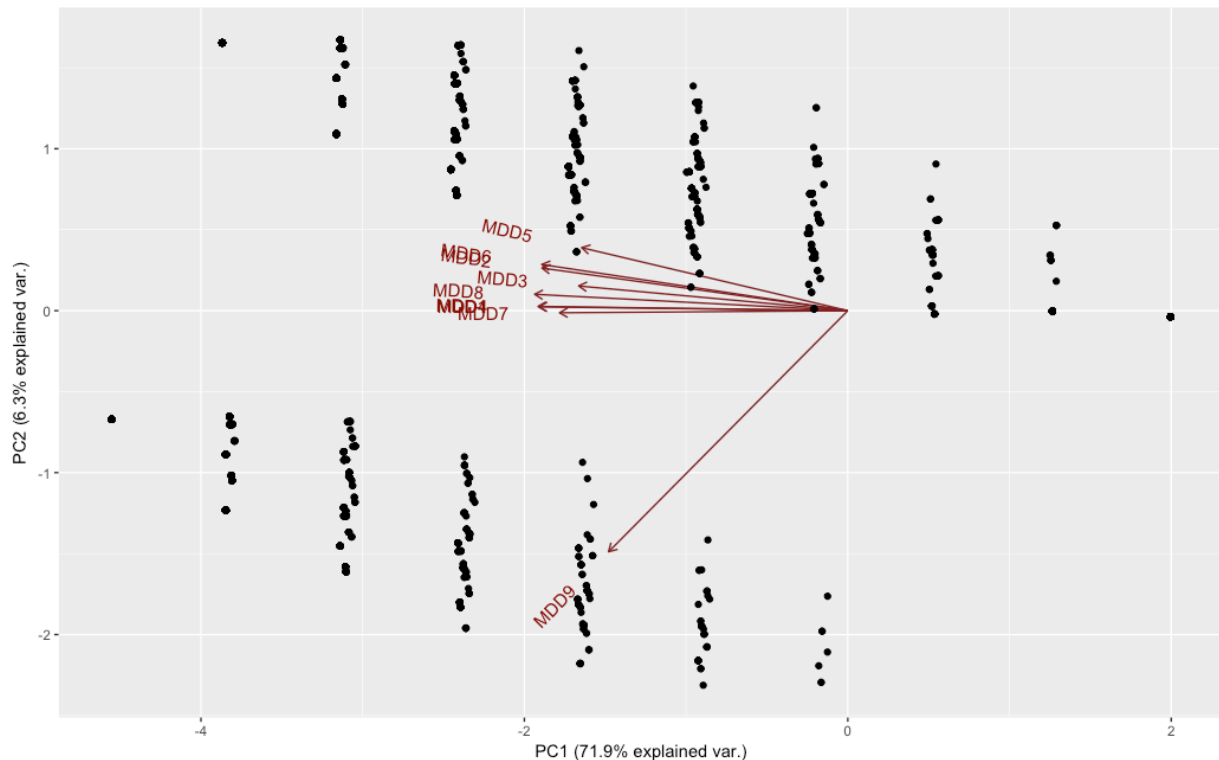


Figure 8-4 Biplots of PCA in PGC-MDD-29.

Datasets are colour coded to show the inherent clustering of datasets by depressive symptoms; MDD1: low mood; MDD2: anhedonia; MDD3: changes in appetite/weight; MDD4: sleep disturbances; MDD5: psychomotor disturbances; MDD6: fatigue; MDD7: feelings of guilt/worthlessness; MDD8: poor concentration and MDD9: suicidality.



8.5.3 Stepwise regression

From a full model with all symptoms of depression and PC1 as above, feelings of guilt/worthlessness is the only significant depressive symptom predictor for T2DM-polygenic scores at $P_T < 0.5$ ($\beta = -0.00004$; $p = 0.0037$; Table 8-3). Feelings of guilt/worthlessness explained only a small amount of the variance in T2DM-polygenic scores at $P_T < 0.5$ ($R^2 = 0.0006\%$). The association remains significant for T2DM-polygenic scores between $P_T < 0.1$ and $P_T < 0.4$, when the PC1-6 from the PGC-MDD 29 meta-analysis, PC1 from the depressive symptoms PCA analysis and an indicator for each study were included as covariates (Table 8-4). Again, the amounts of variance in T2DM-polygenic scores explained by feelings of guilt/worthlessness is very small (R^2 : 0.0006%~0.002%).

Table 8-3 Best-fitting model for T2DM polygenic scores at $P_T < 0.5$ with depressive symptoms in PGC-MDD-29.

β : standardised regression coefficients, * p -value significant at 0.01

Explanatory factors	β	p
PsyCoLaus	0.889	<0.0001*
NESDA	1.694	<0.0001*
SHP0	1.752	<0.0001*
PC1 from depressive symptoms	-0.003	0.1392
Psychomotor disturbances	0.002	0.0868
Feelings of guilt/worthlessness	-0.004	0.0037*

Table 8-4 Association T2DM-polygenic scores and feelings of guilt/worthlessness at various P_T in PGC-MDD-29.

β : standardised regression coefficients; p -value significant at 0.01

P_T	β	R^2 explained (%)	p
<0.0001	0.019	0.0107	0.3712
<0.001	-0.029	0.0247	0.1665
<0.01	-0.020	0.0120	0.0508
<0.1	-0.008	0.0020	0.0054*
<0.2	-0.006	0.0011	0.0051*
<0.3	-0.005	0.0008	0.0017*
<0.4	-0.005	0.0007	0.0016*
<0.5	-0.004	0.0006	0.0016*

8.5.4 Secondary analysis

The stepwise regression was repeated with the sample stratified by i) depression case-control status and ii) sex. T2DM-polygenic scores at $P_T < 0.5$ is the outcome of interest. For depression cases, feelings of guilt/worthlessness is the only significant depressive symptom predictor for T2DM-polygenic scores ($\beta = -0.00005$; $p = 0.0003$; results not shown). For depression controls or males or females, no association between depressive symptom and T2DM-polygenic scores was observed.

8.6 Discussion

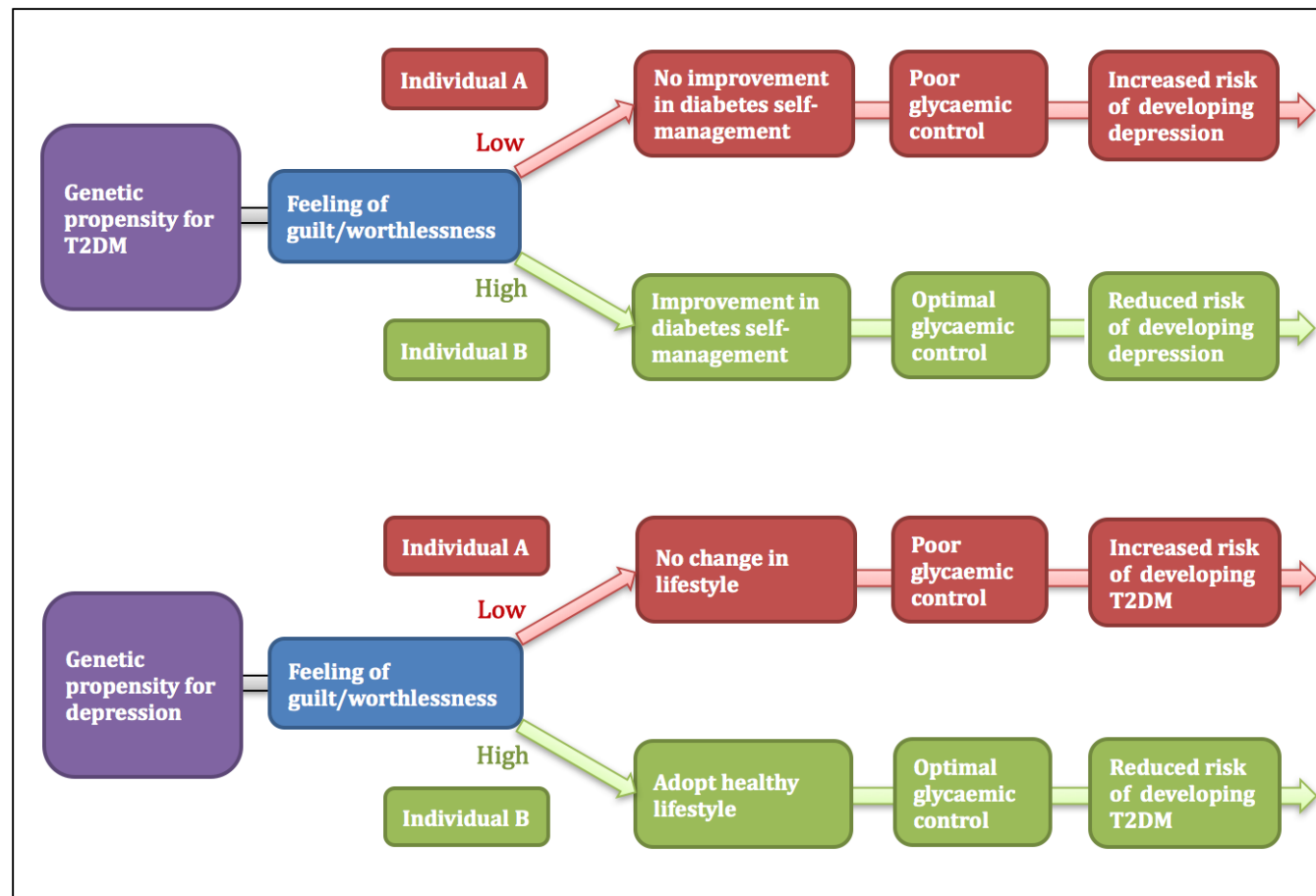
To our knowledge, this is the first study to examine the association between T2DM-polygenic scores and specific depressive symptoms. Our finding suggests that a small, negative but statistically significant association between T2DM-polygenic scores and feelings of guilt/worthlessness, particularly in people with depression. Our finding might appear surprising, given there is no genetic overlap between T2DM and depressive symptoms commonly associated with anthropometric measures, namely changes in appetite/weight, sleep disturbances and fatigue (Twist et al., 2013).

The absence of feelings of guilt/worthlessness might be the mechanism by which a genetic propensity for T2DM exerts its influence on depression, as guilt is a key affective component of conscientiousness (Fayard, Roberts, Robins, & Watson, 2012). Conscientiousness-related traits have been showed to be a predictor of lower BMI (Hampson, Goldberg, Vogt, & Dubanoski, 2007) and BMI is a well-recognised risk factor for T2DM and depression respectively (Kodama et al., 2012; Simon et al., 2006). In people with T2DM, those with high scores for conscientiousness exhibit greater desirable diabetes self-care behaviours than their less conscientious counterparts (Skinner, Bruce, Davis, & Davis, 2014). In people with depression, unhealthy lifestyle-related behaviours,

such as smoking, physical inactivity and poor diet, have been suggested to be risk factors for developing T2DM (Renn et al., 2011).

Our finding is best illustrated if we examine the following two clinical scenarios. Individuals A and B have a genetic propensity to T2DM and both develop T2DM. Individual A might feel less responsible for being diagnosed with T2DM and is therefore less conscientious about diabetes management. In contrast, the feelings of guilt/worthlessness might motivate behavioural changes in individual B. As a result, worse glycaemic control might place individual A at a higher risk of developing depression than individual B, given the reciprocal association between glycaemic control and depression (Schmitz, Deschenes, Burns, & Smith, 2016). This might explain our finding of a negative association between T2DM-polygenic scores and the feelings of guilt/worthlessness. On the other hand, if both individuals develop depression instead of T2DM, an absence of feelings of guilt/worthlessness in individual A might lead to poorer compliance with lifestyle recommendations for depression. For example, overeating might be a mechanism maladapted by individual A to mitigate negative affect, especially in the context of obesity. This, in turn, might have a greater impact on individual A in developing T2DM in comparison to individual B, explaining our finding of a negative association between T2DM-polygenic scores and feelings of guilt/worthlessness in depression cases, but not in controls (Figure 8-5).

Figure 8-5 A theoretical model of the T2DM-depression association, mediated by feelings of guilt/worthlessness, for individual A and B.



Our study suggests certain depressive symptoms might be associated with T2DM, suggesting a depression subtype. The current diagnostic criteria for depression aim to portray it as a homogeneous entity. It might, however, be comprised of multiple heterogeneous phenotypes and present differently in individuals with comorbid medical disorders, such as T2DM. Unrevealing the phenotypic heterogeneity of depression might help the psychiatric field to move forward in the search for its molecular signature.

Replication of our finding is needed before a more robust conclusion can be drawn. The UK Biobank has collected additional data on depressive symptoms, using the Composite International Diagnostic Interview Short Form (Kessler et al., 1998). It should be available in early spring 2017, allowing us to validate our hypothesis and to explore the effect of residual covariates, such as BMI, on the T2DM-depression association. It will also be of interest to examine the genetic overlap between T2DM and number and/or severity of depressive symptoms, allowing us to approach depression from a dimensional perspective. In addition, prospective studies addressing the role of guilt in the T2DM and depression association will help to clarify our finding. A potential implication of our finding for clinicians is the need to consider how a patient's view of their responsibility with T2DM can have an impact on their diabetes self-management. Adopting therapeutic approaches with a personality modification focus might be beneficial in improving diabetes self-care.

8.6.1 Limitation

One major limitation of our study is the method of which we define depressive symptoms with bidirectional response. In this study, depressive symptoms were defined as “absent” or “changes in either direction” which fails to capture the bidirectional nature of those symptoms. Defining those symptoms into four groups (“absence”, “increase”, and both directions “and “reduce”) would lead to a substantial reduction in the number of individuals per group, limiting the power of our

study. We therefore were unable to subdivide the depressive symptoms due to the nature of the information available. This is a major limitation for our study since there is now accumulating evidence of a link between depression and immuno-metabolic dysregulations, with the association being stronger for depressive symptoms being in a specific direction, such as increased appetite/weight and hypersomnia (Cizza et al., 2012; Lamers et al., 2013; Rudolf, Greggersen, Kahl, Hüppe, & Schweiger, 2014). Thus, the bi-directional natures of specific depressive symptoms, namely i) increased/reduced appetite/weight, ii) hypersomnia/insomnia, and iii) psychomotor agitation/retardation, are not captured in this study and their association with T2DM-polygenic scores need to be further explored. Defining a phenotype is a challenge for large-scale, international consortiums, since they are often consisted of various cohorts which use using different diagnostic tools. It can therefore be difficult to combine information at symptoms levels which are compatible across cohorts, leading to some compromise on the quality of the phenotype information.

A further limitation of the study is the collinearity of the depressive symptoms and inherent heterogeneity of the studies included. We have aimed to address this limitation by including study indicators, ancestry informative PCs from the PGC-MDD meta-analysis and depression PCA analysis as covariates in the stepwise regression. A possible way to address both limitations is to re-examine our findings in a larger population-based cohort, such as the UK Biobank when specific depressive symptoms become available. In addition, association also does not imply causation and cross sectional data is used in this study. This is further complicated by the possibility that a subgroup of patients fluctuates between different depressive symptoms. Repeated assessments of depressive symptoms and use of multiple sources would allow a more robust conclusion to be drawn. In addition, objective measures that further explore the feelings of guilt/worthlessness are needed. For example, guilt can be contextual, such as exaggerated responsibility for

uncontrollable events, or generalised, such as “free-floating” guilt which is removed from specific contexts.

8.7 Conclusion

This is the first study to demonstrate a genetic overlap between T2DM and specific depressive symptoms using the polygenic scores approach. Our finding should be interpreted in the context of a small but statistically significant association. The underlying genetic architecture of the T2DM-depression association remains largely unknown. Our findings suggest that the discrepancy in finding between twin studies and GWAS datasets using polygenic score approach might be attributable to the phenotypic heterogeneity of depression, with biologically different subtypes. A better understanding of the mechanisms underlying the T2DM-depression association is of high clinical and scientific relevance.

8.8 Acknowledgments

This research has been conducted using PGC-MDD-29 and the authors would like to thank all participants. CK, KI and CML designed the protocol. CK, NM and DM prepared the dataset for analysis. CK performed the statistical analysis and wrote the manuscript. CML supervised the statistical analysis. All authors reviewed/edited the manuscript. No potential conflicts of interest relevant to this study were reported.

Chapter 9

Conclusion

“Sorrow comes in great waves ... but rolls over us, and though it may almost smother us, it leaves us. And we know that if it is strong, we are stronger, in a smuch as it passes and we remain.”

~ Henry James

9.1 Overview

The aim of this thesis was to examine whether there is a genetic overlap between type 2 diabetes (T2DM) and depression using three complimentary methodologies. In this final chapter, I will first briefly summarise the main findings from the three approaches, followed by an examination of the reasons underlying the discrepancies of the findings, and a conclusion in which future research is discussed.

9.2 Summary of findings



The three complimentary methodologies, namely structural equation modelling in twin datasets, polygenic scores analysis and linkage disequilibrium (LD) score regression in genome-wide association studies (GWAS) datasets are described in detail in chapters 2 and 5. In chapter 3, I explored the genetic overlap between T2DM and depression in two large population-based Scandinavia twin datasets. I found the phenotypic correlation between T2DM and depression was significant in both sexes. In the Swedish sample, this association was primarily attributed to unique environmental effects in males, and genetic effects in females. In the Danish sample, genetic effects accounted for the majority of the covariance in both sexes. In addition, qualitative genetic sex differences are observed in both samples, suggesting different genetic factors are involved across the sexes in explaining the comorbidity. In chapter 4, I examined whether our findings in the Swedish and Danish samples could be generalized to a non-Western population. In the Colombo Twin and Singleton Study-Phase 2 (COTASS-2) sample, the phenotypic correlation between depression and T2DM was significant in females only, and this was mainly due to correlated genetic factors. No qualitative sex differences were observed, suggesting while the same genetic and environmental factors are involved across the sexes, the magnitude of their effect is modulated by sex (quantitative sex differences).

My next focus was on using GWAS datasets to study the relationship between T2DM and depression, as consistency of a finding across different sample populations and methodologies will allow a more robust conclusion to be drawn. In chapter 6, I conducted an exploratory study of the association between i) genetic susceptibility to T2DM and depression case-status and ii) genetic susceptibility to depression and T2DM case-status in the deCODE sample, using polygenic score analysis. T2DM-polygenic scores were not predictive of depression case-status and depression-polygenic scores were not predictive of T2DM case-status. In chapter 7, I evaluated the genetic overlap between T2DM and depression using polygenic score analysis and LD score regression in the UK Biobank sample. I also examined the effect of sex, body mass index (BMI) and severity of depression on the comorbidity. None of the findings were statistically significant after multi-testing correction. In chapter 8, I investigated the shared genetic aetiology between T2DM-polygenic scores and specific depressive symptoms in PGC-MDD-29A small, negative but statistically significant association was observed between T2DM-polygenic scores and the feelings of guilt/worthlessness, particularly in people with depression.

In summary, twin studies reported a genetic overlap between T2DM and depression in both Western (chapter 3) and non-Western (chapter 4) populations. No genetic overlap was observed between T2DM and depression in GWAS datasets using both the polygenic score (chapter 6 and 7) and LD score approaches (chapter 7). Feelings of guilt/worthlessness is the only significant depressive symptom predictor for T2DM-polygenic scores (chapter 8). Our results suggest that although depression is a correlate for T2DM and vice versa as observed in epidemiology studies, there is currently only limited evidence for a shared common genetic effect. Our findings are summarised diagrammatically in Figure 9-1.

Figure 9-1 Summary of findings on the genetic overlap between T2DM and depression by PhD chapters.

R_g : genetic correlation between T2DM and depression from twin studies; r_g : genetic correlation between T2DM and depression from LD score regression; PGS: polygenic scores

Twin studies		 R_g (95% CI)	 R_g (95% CI)
3	Swedish Twin Registry n = 68606 twin individuals	0.06 (-0.13-0.25)	0.23* (0.07-0.38)
		Qualitative sex differences	
		Quantitative sex differences	
3	Danish Twin Registry n = 95403 twin individuals	0.25* (0.23-0.41)	0.18* (0.06-0.31)
		Qualitative sex differences	
		Quantitative sex differences	
4	COTASS-2 n = 9931 twin individuals	0.37 (-0.21-0.93)	0.52* (0.19-0.98)
		Quantitative sex differences	
GWAS studies			
6	DeCODE n = 149838 individuals	1. No association between: <ul style="list-style-type: none"> • T2DM PGS and depression • depression PGS and T2DM Adjusted for year of birth, sex and BMI	
7	Biobank UK n = 152551 individuals	1. No association between: <ul style="list-style-type: none"> • T2DM PGS and depression • depression PGS and T2DM 2. No effect of Sex*PGS or BMI*PGS interaction on T2DM/depression 3. $r_g = -0.08$ (-0.30-0.14), $p = 0.474$	
8	PGC-MDD-29 n = 6542 individuals	1. Association between T2DM PGS and feeling of guilt/worthlessness, particularly in depression cases.	

9.3 The discrepancy in findings between twin studies and GWAS – considerations and critiques

Our findings from twin studies imply that genetic effects are an important contributor in the T2DM-depression comorbidity. It has been observed in females across three studies, and in males in one study. This result was not replicated in three GWAS datasets, but the limited power of GWAS in examining the genetic overlap between T2DM and depression needs to be taken into account. The UK Biobank is the largest GWAS dataset of T2DM and depression to date using the polygenic score approach. My calculations demonstrated that this sample is only powered for detecting a genetic correlation between T2DM and depression of ~ 0.4 (chapter 7). A study which uses the LD score regression approach to estimate 276 genetic correlations among 24 traits from GWA-summary statistics reported a genetic correlation of 0.51 (standard error (SE): 0.08) between depression and schizophrenia and 0.22 (SE: 0.12) for depression and ulcerative colitis (1). Genetic correlations between T2DM and depression derived from GWAS are unlikely to be of the magnitude of ~ 0.4 . From our twin studies, we reported genetic correlations of ~ 0.2 in both the Swedish and Danish samples. The genetic correlations derived from COTASS-2 are of a much higher magnitude; 0.37 (95% CI: -0.21-0.93) for males and 0.52 (95% CI: 0.19-0.98) respectively, but the confidence intervals are very wide. The limited power of the polygenic score analysis is likely to explain in part of our negative findings from the GWAS datasets.

9.3.1 Missing co-heritability

The genetic covariance we observed from twin studies are likely to reflect the upper bound of the variance in liability attributable to genetic factors, given that heritability estimates derived from GWAS and Genome-wide Complex Trait Analysis (GCTA) studies are consistently lower than those from twin studies. The difference has been termed “missing

heritability” (Manolio et al., 2009) and various possible explanations have been postulated to explain this observation including rare variants (Zuk et al., 2014), epigenetics (Furrow et al., 2011) and gene-environment interactions (Kaprio, 2012). It is possible “missing heritability” within traits will also lead to “missing bivariate heritability” or “missing co-heritability”. Most complex traits are considered to be polygenic in nature, with multiple common genetic variants having a small effect size. It is theoretically possible copy-number variations, low frequency and rare variants could explain the genetic architecture of T2DM and depression. This is known as the common disease-rare variant hypothesis (Bodmer & Bonilla, 2008). For T2DM, a recent simulation study has suggested low frequency and rare variants are unlikely to be an important source of contribution to the heritability of T2DM compared to common variants (Fuchsberger et al., 2016). For depression, a rare missense Asn396Ser mutation in the endothelial lipase gene has recently been implicated in the pathogenesis of depressive symptoms in a large population-based cohort (Amin et al., 2016). Thus, the role which low frequency and rare variants might play in the T2DM-depression association remains to be determined. Whole genome sequencing would help to answer this question by identifying rare variants and capturing a few additional common variants, but would not entirely solve the problem. In addition, rare variants are present in the population between 1% and 10% and thus, despite their relatively large effect sizes, the expected effect on population risks is likely to be low (Blanco-Gomez et al., 2016).

It should also be noted heritability estimates derived from GWAS is in the narrow sense, based on the assumption that there is no interaction between alleles and thus, their genetic effects are entirely additive. This assumption might not, however, be justified as research has demonstrated models with genetic interactions (epistasis) can also account for empirical data for medical disorders such as Crohn’s disease (Zuk, Hechter, Sunyaev, & Lander, 2012). Epistasis between the genetic variants already identified from GWAS could therefore potentially in part explain the “missing

heritability“ phenomenon (Zuk et al., 2012). Examining the extent of epistasis in human population studies is, however, difficult and requires large samples (Blanco-Gomez et al., 2016).

Another hypothesis proposed to explain the “missing co-heritability” conundrum is the assumptions underlying the classic twin design might be inaccurate. The equal environment assumption states MZ and DZ twins are correlated for shared environmental influences to the same extent, so the difference in correlation between these two types of twin pairs is solely due to the fact MZ and DZ twins share 100% compared to 50% of their segregating genes. Studies have suggested prenatal developmental processes might influence the epigenetic resemblance of twins, with MZ twins being epigenetically more similar than DZ twins owing to non-genetic causes (van Dongen et al., 2012). This could potentially lead to suboptimal modelling of the common environment component, leading to an overestimation of the heritability estimates (Golan et al., 2014). Further research is needed to clarify this hypothesis and its effects, especially for T2DM and depression.

The “missing co-heritability” that we observed in the T2DM-depression association could also in part be explained by the genetic effect of an intermediate phenotype affecting the two main traits. Visceral obesity and BMI has been postulated to lie on the causal pathway between T2DM and depression. It is therefore possible that obesity-related genetic effects might be involved in the genetic overlap between T2DM and depression. For example, a gene which influences obesity will promote a systemic low-grade inflammation that could contribute to the development of both T2DM and depression. Obesity might therefore act as a heritable intermediate trait conferring risk to both disorders. “Mediated pleiotropy” is the term used to describe the concept when a genetic variant is linked to a complex phenotype because it is associated with an intermediate phenotype that is causally related to the complex phenotype (Solovieff, Cotsapas, Lee, Purcell, & Smoller, 2013). In this thesis, I did not examine

the effect of obesity-polygenic scores on the T2DM-depression association. Future research could clarify the complex relationship between T2DM, depression and BMI. I attempted to examine the potential effect of BMI on the genetic overlap between T2DM and depression in the deCODE and UK Biobank samples (chapter 6 and 7), but no conclusive conclusion could be drawn due to the limited power of the studies. In addition, BMI was measured at the point of data collection, and no information was available to assess its temporal relation to the onset of T2DM and depression. It is therefore possible common genetic signatures and biological pathways might still mediate the T2DM and depression link, despite our inconclusive findings.

9.3.2 Heterogeneity of depression

Differences in case ascertain for depression should be considered when interpreting our results. For the twin studies, the sources of case definition differ between the studies. Hospital registries were used in both the Swedish and Danish twin registries whereas self-reported questionnaire (Beck Depression Inventory) was used in the Colombo Twin and Singleton Study Phase 2. The Beck Depression Inventory was developed to measure the severity of depression, with participants being asked to rate how they have been feeling for the preceding two weeks (Beck et al., 1996). It therefore captures a different timeframe (current presentation) to the definition used in the Swedish and Danish twin registries (previous/current diagnosis of depression at the time of data retrieval).

For the polygenic scores analysis, the depression phenotype also differs across the studies. In deCODE, it is defined by primary diagnosis of lifetime depression using structured diagnostic interview. In the UK Biobank, it is a combination of primary diagnosis of depression using inpatient hospital episode data and previous visit to a General Practitioners/psychiatrist for stress, anxiety or depression, and at least one period of depression/anhedonia lasting at least two weeks using self-

reported questionnaire. It therefore captures both current presentation and lifetime diagnosis of depression. In the PGC-MDD-29 sample, four datasets were used in the analysis. They all defined lifetime diagnosis of depression using structured diagnostic interview but different tools were used; Mini Neuropsychiatric Diagnostic Interview 6.0 (Sheehan et al., 1998) for CoFaMS, Diagnostic Interview for Genetic Studies (Nurnberger et al., 1994) for PsyCoLaus, Composite Interview Diagnostic Instrument (Wittchen, 1994) for NESDA and Mini-Mental Test (Folstein et al., 1975) for SHP0. The tools vary in their reliability in eliciting information to support this designation.

In addition, there are variations in the diagnostic criteria for major depressive disorder between diagnostic classifications, namely the International Classification of Disease and Diagnostic and Statistical Manual of Mental Disorder. Criteria for major depressive disorder have also changed over time and different editions of the diagnostic classification were used to define depression in this thesis. Epidemiology studies have also consistently demonstrated discrepancies between hospital registry data, structured diagnostic interview and self-report questionnaire in the diagnosis of depression. Variations in the definition of depression in this thesis therefore limit to a certain extent the generalisability of our findings.

Depression is a difficult phenotype to define, given the overt reliance on subjective information for its diagnosis and the heterogeneity observed in both its clinical presentation and trajectory and the lack of a specific biomarker. What has remained constant is that depression is a syndrome defined by disturbances across multiple domains i) mood, ii) anhedonia (a loss of interest or pleasure in daily activities), iii) changes in appetite/weight, iv) sleep disturbances, v) psychomotor disturbances, vi) fatigue, vii) feelings of guilt/worthlessness, viii) poor concentration and ix) suicidality. The quality of the phenotyping is sometimes compromised in

genetic epidemiology in exchange for large sample sizes, in order for a study to have adequate power in to test specific hypothesis.

9.3.3 Advances in GWAS

The base dataset used to create T2DM polygenic scores was the summary statistics of stage 1 of DIAGRAM version 3 (Morris et al., 2012) and it is used in chapters 6, 7 and 8. Two different base datasets were used to create depression polygenic scores, namely the summary statistics from PGC-MDD-1 and PGC-MDD-29 (Ripke et al., 2013). The PGC-MDD-1 has 9240 depression cases and 9519 controls whereas PGC-MDD-29 study has 16823 depression cases and 25632 controls (Ripke et al., 2013). PGC-MDD-1 was used in chapter 6 and PGC-MDD-29 was used in chapter 7. The reason why different base datasets were used was because the analysis for chapter 6 was conducted in summer 2015 and PGC-MDD-29 was not publically available during that period. The PGC-MDD-29 is a larger cohort, with more depression cases and controls. The polygenic scores from PGC-MDD-1 explain 0.20% of the variance in depression whereas the scores from PGC-MDD-29 explain 1.9%. This difference will have an impact on the power of the respective study in detecting the association between T2DM and depression polygenic scores.

9.4 Clinical implication and future direction

This thesis has moved the field forward by establishing a body of valuable knowledge into two conditions which are among the most important burdens on health and wellbeing globally. The clinical implication is hard to fully foreshadow at present, given the discrepancy in our findings between twin studies and GWAS. If the genetic overlap between T2DM and depression as observed in twin studies is replicated in GWAS, the clinical implication might lie in genetic risk prediction. It has the potential to identify individuals with T2DM who are at high-risk for depression, allowing appropriate intervention to be recommended in a timely manner (Wray et al., 2013). In addition, genetic profiling may help

to classify treatment responders for individuals with T2DM and depression, allowing clinicians to treat them accordingly. This, in turn, will reduce the costs of unnecessary exposure to medication for an individual. At present, the amount of variance predicted by polygenic scores is moderate and probabilistic. Yet, with increasingly large sample and international collaboration, single nucleotide polymorphisms associated with depression have been identified; from no significant finding in the PGC-MDD phase 1 study (9240 depression cases) (Ripke et al., 2013), to 15 loci in an analysis of self-reported depression (75607 depression cases) (Hyde et al., 2016) and 44 loci in the latest PGC-MDD study (130664 depression cases) (Wray & Sullivan, 2017). This in turn will improve the quality of the polygenic scores for depression.

From a clinical intervention perspective, a useful avenue might lie in developing special tailored depression programme for people with T2DM. For it to be effective in improving both depression and type 2 diabetes outcomes, a multidisciplinary approach is required. For example, a patient with T2DM who is at risk of depression might benefit from receiving cognitive behavioural therapy to recognise negative core beliefs and malfunction thinking styles, while occupational therapy will enable behavioural activation and reduce social isolation. In addition, given the complex interplay between weight, T2DM and depression, involvement of dietician for adopting healthy eating habits will be a useful addition. From a public health perspective, National Institute for Health and Clinical Excellence guideline already recommends regular depression screening for individual with T2DM. This role currently mainly lies in the remit of practitioners working in primary care and general hospital settings. Raising public awareness and educating multidisciplinary team that patients with a chronic physical health problem such as T2DM are at a high risk of depression, particularly where there is functional impairment, is essential. In addition, regular diabetes screening for people with depression will be a useful addition, addressing the discrepancy between physical and mental health.

Multiple environmental factors have been identified to influence the T2DM-depression association including stress, physical exercise, diet and lifestyle. It is possible the interaction between genetic and environmental factors can influence the progression and pathogenesis of T2DM and depression. Gene-environment interaction is an area that needs to be explored in understanding the T2DM-depression association, given genotypes can affect an individual's responses to the environment; whereas environment can differentially affect the expression of genotypes. In addition, research, which takes into account the epigenetic effects on the risk of complex traits, might hold the key to unlocking the T2DM-depression association.

Insulin resistance is on the causal pathway of developing T2DM and a meta-analysis has demonstrated a small but statistically significant association between insulin resistance and depression (Kan et al., 2013). We have postulated a theoretical model whereby the absence of feelings of guilt/worthlessness might be the mechanism by which a genetic propensity for T2DM exerts its influence on depression (chapter 8). A similar finding between a genetic propensity for insulin resistance and the feelings of guilt/worthlessness in individuals with depression will provide evidence for the hypothesis, allowing further development of a biomedical model of for the T2DM-depression association.

The current clinical diagnostic boundaries for depression might not reflect the underlying psychopathology given the extent of its phenotypic heterogeneity (Harald & Gordon, 2012). There is accumulating evidence for developing cross-diagnostic treatment paradigms in psychiatry (Anttila et al., 2016). Advances in psychiatric genetics have expanded our understanding of the biological underpinning of psychiatric disorders, and a review of the current classification of depression might inform our understanding of its association with T2DM.

9.5 Conclusion

Studying the genetic overlap between T2DM and depression could help us to unravel their underlying pathophysiology, and explain the observed epidemiological association. Future advances in statistical genetics to handle the “missing heritability” conundrum, together with larger samples of whole genome sequencing data, has the potential to fully disentangle the complex T2DM-depression association.

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